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THE 12th INTERNATIONAL RAPESEED CONGRESS

V

Sustainable Development in Cruciferous Oilseed Crops Production

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QUALITY, NUTRITION, PROCESSING AND TRADE

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GCIRC and GCIRC Congress

The Groupe Consultatif International de Recherche sur le Colza (GCIRC), with its office at 12, avenue George V, 75008 Paris, France, was established to encourage scientific and technical research concerning the improvement of rapeseed and its processed products from the agronomic, technological and foods perspectives, and to encourage collaboration among researchers. The organization has no political activity.

The GCIRC is managed by the Board of Directors elected by 70 subscribing members from more than twenty countries. The secretary of the GCIRC is Mr. André Pouzet, CETIOM, with office support of Mrs. Laurencine Lot. Professor Fu Tingdong, Huazhong Agricultural University, Wuhan 430070, China is the current GCIRC President.

The GCIRC International Rapeseed Congress is held every four years with GCIRC Technical Meetings between two major events. Staring from 1987, GCIRC issues an award on its every congress to honor scientists who have made significant contributions to one of the areas in rapeseed research. So far there are six scientists having received the honor: Dr. Baldur R. Stefansson (Canada, 1987), Prof. FU Tingdong (China, 1991), Dr. R. Keith DOWNEY (Canada, 1995), Dr. Jacques MORICE (France, 1995), Dr. Gerhard RÖBBELEN (Germany, 1999), Dr. Jan KRZYMANSKI(Poland, 2003).

The 12th International Rapeseed Congress, with the theme of "Sustainable Development in Cruciferous Oilseed Crops Production" is held in Wuhan, China, March 26-30, 2007. The congress consists of a program with the following seven sections: Genetics and Breeding, Biotechnology, Agronomy, Plant Protection, Quality, Nutrition and Processing, Feed and Industrial Raw Materials, and Trading and Policies. Each of these sections is divided into oral and poster presentations. In addition, the congress also hosts 8 workshops covering a range of topics.

The contributions from oral presentations and posters are included in books of Abstracts and Proceedings with the full papers, and an electronic disc.

On the occasion of the 20 anniversary of the founding and awarding of the Eminent Scientist Award by GCIRC, Ministry of Agriculture of the People's Republic of China will grant an honored certificate to all the awardees ever on this congress.

The 12th International Rapeseed Congress

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Welcome Address by the President at the Opening Ceremony of the 12th International Rapeseed Congress

Good morning ladies and gentlemen,

On behalf of GCIRC, the Advisory Committee, the Organizing Committee, the Scientific Committee, the International Scientific Committee of the 12th International Rapeseed Congress and all the staff members of the Congress, it is a great honor for me at this significant moment to extend our warmest welcome to the friends and participants from all over the world. I would like to take the opportunity to express our gratitude to the Ministry of Agriculture of the People's Republic of China, Hubei Provincial Government, Wuhan Municipal Government, the Chinese Academy of Engineering and all the other supporters. My gratitude also goes to the enterprises that have sponsored the Congress.

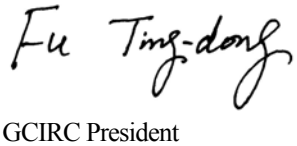
2007 is the year of the 20th anniversary of the GCIRC Eminent Scientist Award, which is a particular honor for the distinguished scientists in rapeseed research field. Six scientists so far have won the prize. Today, in addition to myself, we are privileged to have Dr. K. Downey, Dr. G. Röbbelen, Dr. J. Krzymanski presented on the Congress. I would like to express our warmest welcome to them.

As you all know, China is the country with the largest rapeseed planting area, which is about 7 million hectares, with 11 million tons of production annually, of which 85% are produced in Yangtze River Basin. Rapeseed yield in China accounts for 30% of the world's total production. Hubei, located in the middle of the Yangtze River Basin, is the largest rapeseed production province. Two major rapeseed research institutes in China, Huazhong Agricultural University and the Oil Crop Research Institute of Chinese Academy of Agricultural Sciences, and the National Research Center of Rapeseed Engineering Technology, jointly-run by the two institutes, are located in Wuhan, the capital city of Hubei province. I believe that the 12th International Rapeseed Congress will have a great impact on both rapeseed production and the research in China, particularly in Hubei Province. It is hopeful that the Congress will be a very good occasion for all our participants to discuss, communicate and learn from each other in the field of rapeseed research and production.

In retrospect, the history of rapeseed development has witnessed the significant achievements of rapeseed research in the past 30 years. Scientists all over the world have contributed jointly to the development in quality improvement, hybrid vigor, resistance breeding, biotechnology, cultivation physiology *etc*. Rapeseed is not only an edible oil crop, a fertilizing crop, or a honey crop, but also a major promising biodiesel crop, which brings about new opportunities for rapeseed production and research. The GCIRC has been the active force in promoting the exchange, the dissemination and the utilization of the achievements.

The theme of this Congress is "The Sustainable Development in Cruciferous Oilseed Crops Production". Seven Hundred abstracts related to the theme have been submitted and more than 700 scholars are attending the Congress, which demonstrates your solicitude, support and wish for the 12th International Rapeseed Congress held in Asia for the first time. I am convinced that the 12th International Rapeseed Congress will be worth your trust.

To end my speech, I wish every participant a pleasant stay in Wuhan, and the Congress a brilliant success. Thank you.



GCIRC President
President of the 12th International Rapeseed Congress
Professor of Huazhong Agricultural University

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Quality Analysis and Nutrition

Rapeseed for human nutrition – present knowledge and future options

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Brassica species are now the second largest oilseed crop worldwide yielding an edible oil with remarkable nutritional attributes.

Fatty Acids. Nutrition strategy for prevention of cardio-vascular diease aims at reducing intake of saturated and raising consumption of n-3 polyunsaturated and mono-unsaturated fatty acids. At the same time moderation of n-6 polyunsaturated fatty acids is advised. These 4 targets can be reached by raising the consumption of rape oil.

Rape oil has an exceptionally low amount of saturated fatty acids and a comparatively low concentration of n-6 fatty acids (linoleic around 20 %). (Table 1).

Table 1. Fatty acid content (g/ 100g total fatty acids). of edble vegetable fats and oils (Gurr, 1992)

Commodity	Saturated Fatty Acids	Monounsaturated Fatty Acids	Linoleic Acid (N-6)	Linolenic Acid (N-3)
Coconut	90	7	2	0
Corn	17	30	50	2
Olive	14	72	11	1
Palm	47	43	8	trace
Rape (low erucic)	5	54	23	10
Safflower (high linoleic)	9	15	73	1
Soyabean	14	25	52	7
Sunflower	12	33	52	trace

Simultaneously, the ratio of dietary n-6 / n-3 polyunsaturated fatty acids from presently 15: 1 in the Western diet can be lowered towards 5:1 as recommended by international recommendations for a healthy diet (German Nutrition Society, 2002; Krauss *et al.*, 2000). This is due to the comparatively low ratio of n-6/ n-3 in rape oil (Table 2).

Table 2. Comparison of edible oils: n-6 and n-3 fatty acids in absolute and relative contents.

Commodity	n- 3 Fatty acids g / 100g	Ratio n-6 / n-3
Marine Fish (Herring)	3	0.1
Nuts (Walnut)	3	10
Rape Oil	11	1.9
Linseed Oil	57	0.28
Soy Oil	7	7.7
Olive Oil	< 1	13.2
Sun Flower Oil	< 1	> 70

Considering these data it is no surprise that diverse beneficial metabolic effects of rapeseed oil have been reported. In controlled studies with healthy adult volunteers this oil provoked favourable effects on level and composition of serum lipoproteins (Kratz *et al.*, 2002). First, the serum lipoprotein profile changes were as favourably as those caused by olive or sunflower oil. Secondly, the Low Density Lipoprotein (LDL) particles were particularly protected from oxidation presumably by the high content of the monounsaturated fatty acid, oleic acid (18:1), in the lipoprotein following rapeseed oil consumption. Third, a rise of eicosapentanoic acid (20:5, n-3) in these particles (Kratz et al., 2002) proved the elongation of α- linolenic acid to this long-chain n-3 fatty acid which has been shown repeatedly to be protective what concerns cardio-vascular events (Mozzafarian, 2006).

Moreover, most of the prospective epidemiological studies suggest that the preeminent n-3 polyunsaturated fatty acid in rapeseed oil, namely α-linolenic acid, reduces the incidence of coronary heart disease (Brouwer IA, 2004). In an intervention study with patients after myocardial infarction in which the n-6 / n-3 ratio of dietary fatty acids (linoleic vs linolenic acid) was about 4 : 1, a significant reduction in the reinfarction rate has been achieved (de Lorgeril *et al.*, 1999). In summary, a multitude of data strongly suggest a cardioprotective effect of rapeseed oil by its dominant n-3 polyunsaturated fatty acid α-linolenic acid (18:3, n-3) which warrants to be corroborated by further confirmatory evidence (Barth, 2006; Geleijnse, 2006; Zock, 2006).

Safety. Whereas n-3 fatty acid containing food, as e. g. marine fish, has generally been reported to lower the risk for cancer some earlier studies observed a rise of risk for prostate cancer. These latter studies are inconsistent, because two prospective and four case-control studies observed no association between dietary, serum, adipose, or prostate tissue α-linolenic acid levels and prostate cancer risk, whereas, in contrast, three prospective and five case-control studies reported a statistically significant or non-significant 2-4-fold increased risk of prostate cancer among men with high α-linolenic acid

exposure determined by dietary or blood assessment. All these studies are merely epidemiological evidence, whereas *in vitro* and metabolic findings are lacking (Leitzmann, 2006; Leitzmann, 2004).The lack of consistency may be caused methodologically as exposure to α-linolenic acid can hardly or only imprecisely determined in epidemiological studies (Leitzmann, 2006). In summary, further evidence in men is needed to resolve the relation of n-3 fatty acids, α-linolenic acid in particular, and risk of prostate cancer (Leitzmann, 2006). To summarize, there is no consistent set of data from epidemiological, metabolic and biochemical research substantiating a risk of rape oil consumption at the present time. Further such research is needed. Another topic for future research is the definition of safe limits of different procedures applied in industrial usage of rapeseed oil, particularly heating procedures like deep frying and others with the aim to prevent or minimize formation of potentially toxic products (Warner, 1999).

Rapeseed Protein. Animal nutritionists are well aware of the nutritional value of rapeseed protein. It is extensively used in monogastric, poultry and ruminant feeds. For example, satisfying weight gains of more than 800 g/day can be achieved in pig production if 10 % of rapeseed meal with less than 10 mmol glucosinolates/kg meal is fed (Weiss, 2006).Nutritional evaluation of rapeseed meal by earlier investigators resulted in somewhat lower but considerable weight gains in several animal species if compared to soy concentrate (Fig.1). This is based on the high biological value of rapeseed protein caused by a favourable amino acid profile (O´Mara, 1997). The somewhat lower weight gain by rapeseed meal as compared to soy concentrate is caused by a lower true protein digestibility (78.9% vs 90.4%) (Drouliscos, 1969) and bioavailability of the limiting amino acid lysine. The prececal digestibility of rapeseed meal lysine as measured in the pig amounts to 75 % as compared to 89 % of soy concentrate lysine (Weiss, 2006). Also the PDCAAS was reported to be significantly lower (Rozan, 1997). This can be explained by the comparatively high concentration of 14-16 % of crude fiber (per dry matter) in rapeseed meal impeding protein digestibility. So, whereas the content of 5,9 g lysine per 16 g N of rapeseed protein is considerable and sufficient to meet the nutritional requirements this satisfying biological value is compromised by the accompanying fiber. This leads to conclude that attempts to exploit rapeseed protein for human nutrition have to remove substances impeding digestibility either by breeding or food technology (see below).

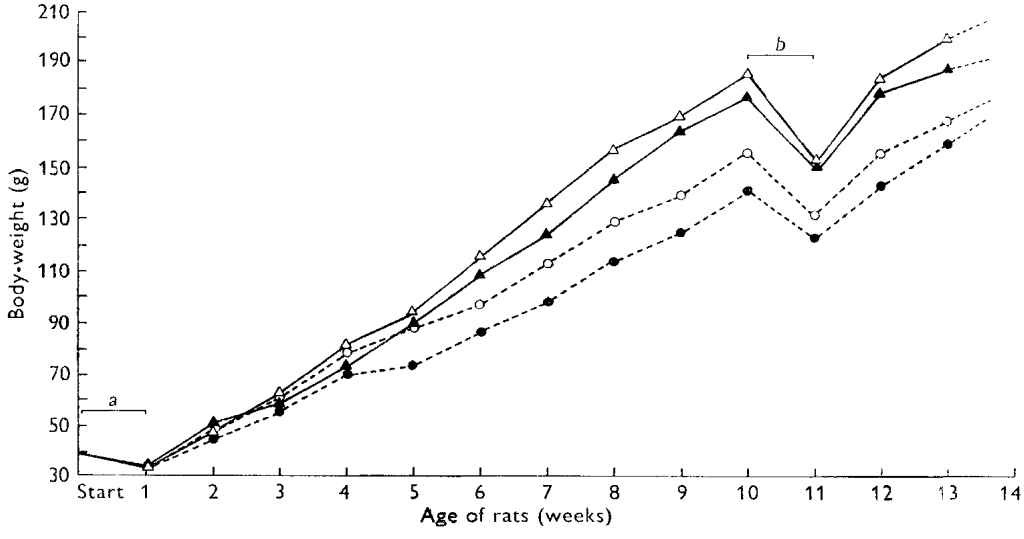

Fig. 1. Body-weight (g) gains of rats from weaning to maturity. The periods of protein-free feeding are indicated by *a* and *b* on the curve. △—△, SM diet; ▲—▲, CS diet; ○—○, RM(S) diet; ●—●, RM(PS) diet.

Fig. 1: RM(S) = rapeseed meal. SM = Soy concentrate.

Drouliscos (1969)

Minor components. Rapeseed meal contains water-soluble vitamins, as thiamine (0,82 mg/100 g dry wt), riboflavin (0,33 mg/100g), niacin (8 mg/100g), pyridoxine (1,8 mg/100g) and pantothenate (0,48 mg/100g) (Mansour; 1993) Tocopherols range between 600-700 mg/kg rapeseed oil, γ- and α- forms contributing 64 and 35 %, respectively, and the δ-form less than 1 % (Goffman, 2000). Rapesseed oil has the highest amount of total antioxidants, stability (against oxidative damage), and total radical-trapping antioxidant potential among other oils (sunflower varieties and grapeseed) (Ciz, 2002) A number of more polar phenolics, like sinapinic acid and its derivatives contribute to this activity (Vuorela, 2005).

Technology. As mentioned the prerequisite for rapeseed protein use in human nutrition is the removal of antinutrients, as glucosinolates, tannins and fiber among others. Several technological attempts are being pursued to reach preparations with satisfying solubility and sensoric quality for food purposes (Anonymous, 2006; Klockemann, 1997; Leckband, 2002).

Breeding. A major breeding target is the creation of yellow-seeded varieties with a lower amount of substances as sinapine**,** cellulose, tannins or lignine in order to raise protein digestibility and sensoric acceptance. A health-related target is the introduction of " fish" very long-chain n-3 polyunsaturated fatty acids (20:5, n-3 and 22:6,.n-3) (Leckband, 2002) for populations living in areas distant from the coast. Moreover, plant production of these nutritionally valuable fatty acids will be an important alternaive to the insufficient if not shrinking marine resources for future world nutrition (Pimentel, 1996).

Summary and conclusions. Rapeseed oil is a nutritionally highly valuable complement of human nutrition recommended unanimously by numerous national and international authorities because of its low content of saturated and high content of n-3 polyunsaturated and monounsaturated fatty acids. Recent observations suggesting a possible relation with prostate disease and risks by heating this commodity by industrial processes or cooking should be pursued and scrutinized in future research. It is worthwile to elaborate further by nutrition research the health attributes of minor compounds (vitamins, polyphenols, plant sterols among others) of rapeseed. The outstanding antioxidative potential of these constituents harbors a promising perspective for human health. Rapeseed protein is a biologically valuable plant protein with an optimal perspective for human nutrition provided antinutrients impeding protein digestibility will be removed by breeding or food technology. Plant breeding offers numerous options for future usage of rapeseed. Among these is the introduction of very-long-chain n-3 polyunsaturated fatty acids of the seafish type into rapeseed oil particularly attractive for human health.

References

Anonymous. (2006). Burcon nutrascience corp.www.burcon.net; accessed 28. 11. 2006

Barth, C. Erbersdobler., HF. (2006). Cardiovascular health and safety of rapeseed oil in human nutrition. *Akt. Ernaehr Med, 31*, 2111-2117.

Brouwer IA, Katan. M., Zock PL. (2004). Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: A meta-analysis. *J Nutr, 134,919-922*

Ciz, M., Gorinstein, S, Lojek, A, Martin-Belloso, O, Leontowich, H Trakhtenberg, S. (2002). Comparative antioxidative properties of selected seed oils. *Free Radical Research, 36 S1*, 64-65.

de Lorgeril, M., Salen, P., Martin, J. L., Monjaud, I., Delaye, J., & Mamelle, N. (1999). Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: Final report of the Lyon diet heart study. *Circulation, 99*(6), 779-785.

Drouliscos, N., Bowland,JP. (1969). Biological evaluation of rape-seed meal in rats. *Br J Nutr, 23*, 113-118.

Geleijnse, J. (2006). Health effects of alpha-linolenic acid and experimental design of the alpha omega trial (abstr.). *Akt Eraehr Med, 31*, 214-215.

German, S. N. (2002). *Reference values for nutrient intake*. Frankfurt/Main, Germany: Umschau Braus.

Goffman; FD, Moeller., C, &. (2000). Changes in tocopherol and plastochromanol-8 contents in seeds
and oil of oilseed rape (brassica napus l.) during storage as
influenced by temperature and air oxygen. *J Agric Food Chem, 48*, 1605-1609.

Gurr, M. I. (1992). Role of fats in food and nutrition. Elsevier Applied Science Publishers, London.

Klockemann, D., Toledo, R, Sims; KA. (1997). Isolation and characterization of defatted canola meal protein. *J Agric Food Chem, 45*(10), 3867-3870.

Kratz, M., Cullen, P., Kannenberg, F., Kassner, A., Fobker, M., Abuja, P. M., et al. (2002). Effects of dietary fatty acids on the composition and oxidizability of low-density lipoprotein. *Eur J Clin Nutr, 56*(1), 72-81.

Krauss, R. M., Eckel, R. H., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., et al. (2000). AHA dietary guidelines: Revision 2000: A statement for healthcare professionals from the nutrition committee of the american heart association. *Circulation, 102*(18), 2284-2299.

Leckband, G., Frauen, M, Friedt,W. (2002). Napus 2000. Rapeseed (brassica napus) breeeding for improved human nutrition. *Food Res Int, 35*(2-3), 273-278.

Leitzmann, M. (2006). N-3 fatty acids and risk of prostate cancer (abstr.). *Akt Eraehr Med, 31*, 215.

Leitzmann MF, S. M., Michaud DS, Augustsson K, Colditz GC, Willett WC, Giovannucci EL. (2004). Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer. *Am J Clin Nutr., 80*, 204-216.

Mansour; EH, D., E, Lugasi, A, Gaal, O Barna, E, Gergeley,A. (1993). Effect of processing on the antinutritive factors and nutritive value of rapeseed products. *Food Chemistry, 47*, 247-252.

Mozzafarian, D. R., Rimm, EB. (2006). Fish intake, contaminants, and huiman health. *JAMA, 296*, 1885-1899.

O´Mara, F., Murphy, JJ, Rath, M. (1997). The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. *J Anim Sci, 75*, 1941-1949.

Pimentel, D., Shanks, RE, Rylander, JC. (1996). *Energy use in fish and aquacultural production*. In: Food, Energy, and Society. Pimentel,D and Pimentel, M ed. Niwot,Co: University Press of Colorado.

Rozan, P., Lamghari, R, Linder, M, Villaume, C, Fanni, J, Parmentier, M, Mejean, L. (1997). In vivo and in vitro digestibility of soybean, lupine, and rapeseed meal proteins after various technological processes. *J Agric Food Chem, 45*, 1762-1769.

Vuorela, S., Kreander, K, Karonen, M, Nieminen, R, Hämäläinen, M, Galkin, A, Laitinen, L, Salminen, J, Moilanen, E, Pihlaja, K, Vuorela, H, Vuorela, P, Heinonen, M. (2005). Preclinical evaluation of rapeseed, raspberry, and pine bark phenolics for health related effects. *J Agric Food Chem, 53*, 5922-5931.

Warner, K. (1999). Impact of high-temperature food processing on fats and oils. *Adv Exp Med Biol, 459*, 67-77.

Weiss, J. Schoene., F. (2006). Rapsextraktionsschrot in der Schweinefütterung. *Praxisinformation*. UFOP. www,ufop.de; accessed 28. 11. 2006

Zock, P. (2006). An overview on alpha-linolenic acid and cardiovascular disease (abstr.). *Akt. Eraehr Med, 31*, 214.

Recent advances in glucosinolate research

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Abstract

This contribution will review recent advantages in glucosinolate research based on own research projects and literature data. Glucosinolates were investigated comprehensively in the early 1980s when high glucosinolate contents in rapeseed meal caused problems in animal nutrition and double low oilseed rape varieties were introduced. Since then the focus of research has changed in such way that nowadays metabolism and transport of glucosinolates, ecological relevance of glucosinolates and practical applications in bio-fumigation and use as health promoting substances are the main topics of interest.

Investigations were undertaken to resolve the metabolic background of lower glucosinolate contents in double low oilseed rape varieties in comparison to single lows. Earlier studies have already shown that double low varieties accumulate sulphur in pod walls and it was proposed that a metabolic block exists which led to the accumulation of intermediary products of the glucosinolates biosynthesis which could not be transported into seeds. Recently conducted field trials support this hypothesis. Double low varieties showed distinctly higher total sulphur contents in pod walls at seed maturity than single lows where a higher proportion of sulphur was bound in seeds. During pod development an early accumulation of glucosinolates in seeds took place. Only low glucosinolate contents of <2.5 µmol g^{-1} d.w. could be determined in pod walls of single low varieties. In double low varieties the glucosinolate content in pod walls decreased from more than 30 µmol g^{-1} d.w. at the beginning of pod development to about 1µmol g^{-1} at seed maturity.

Bio-fumigation as a technique to reduce biologically soil-borne infestations with nematodes and fungi is discussed as well as effects of glucosinolates on different insect populations in relation to the sulphur nutritional status.

Health promoting effects of glucosinolates were investigated in experiments with piglets where seeds of *Tropaeolum majus* were fed in different amounts and growth parameters such as animal weight and fodder intake, chemical data like the isothiocyanate content and pH in urine, and microbial investigations of the excrements were recorded. In human nutrition efforts were undertaken to enrich the glucoraphanine and sulphoraphane content in *Brassica oleracea* which are supposedly have a highly anti-carcinogenic potential.

Key words: single/double low oilseed rape, pod wall, biofumigation, health promoting effect

Introduction

In the 1980s and 1990s research in the field of glucosinolates (GSL) focused on the biosynthesis and breeding of varieties, which were low in seed GSLs in order to avoid a thyroid effect when using rapeseed meal in animal nutrition. Besides environmental and agronomic factors, the physiological functions of GSLs were investigated comprehensively (Schnug, 1990, 1993). Since the introduction of double low oilseed rape varieties in the 80´s the physiological background of a lower GSL content in seeds of double low varieties is still unknown. Single low and double low oilseed rape varieties contain similar amounts of total S in vegetative plant parts, while seeds show significantly lower S and GSL contents (Fieldsend and Milford, 1994). There exist opposing theories how these differences can be explained. It is commonly accepted that the seed-GSLs are mainly synthesized in the pod walls (De March et al., 1989). The lower GSL content in double low varieties is explained either by a metabolic block in GSL biosynthesis or by a reduced transport of GSLs or intermediary products of GSL biosynthesis into seeds.

Today the main focus in GSL research is put on application aspects. Efforts are undertaken to take advantage of the biocidal effect of GSLs in agriculture. “Biofumigation” is a term which refers to the release of biocidal compounds from GSL-containing crops during green manure, crop rotation or direct application for example as pellets, which aims at a suppression of soil-borne pests and pathogens (Sarwar et al., 1998; Lazzeri et al., 2004). Additionally research is carried out on the health promoting effects of GSLs and so-called nutraceuticals which contain high contents of health promoting substances (Mithen et al., 2000; Moreno et al., 2006). It is the aim of this contribution to summarize recent advances in GSL research.

Glucosinolates in developing seeds and pod walls of single low and double low oilseed rape varieties

An investigation of the changes in total sulfur (S), sulfate-S and GSL contents of developing seeds and pod walls of a single low and double low winter oilseed rape variety provided new insight into GSL biosynthesis of oilseed rape (Bloem et al., 2007). At the very start of pod development, differences in S accumulation in whole pods were not significant between the two varieties, but already at BBCH 73 a higher proportion of S was bound in seeds of the single low variety (*Jet Neuf*), while in the double low variety (*Ceres*) a higher proportion was bound in pod walls (Fig. 1). Nevertheless it could be shown that no differences in GSL biosynthesis could be observed until BBCH 77. Afterwards a strong increase in seed GSL occurred in the single low variety, while the double low variety remained stable with respect to seed GSLs and free sulfate accumulated in pod walls. As a result the single low variety bonded the same amount of total S in seeds at maturity as the double low variety did in

the pod walls.

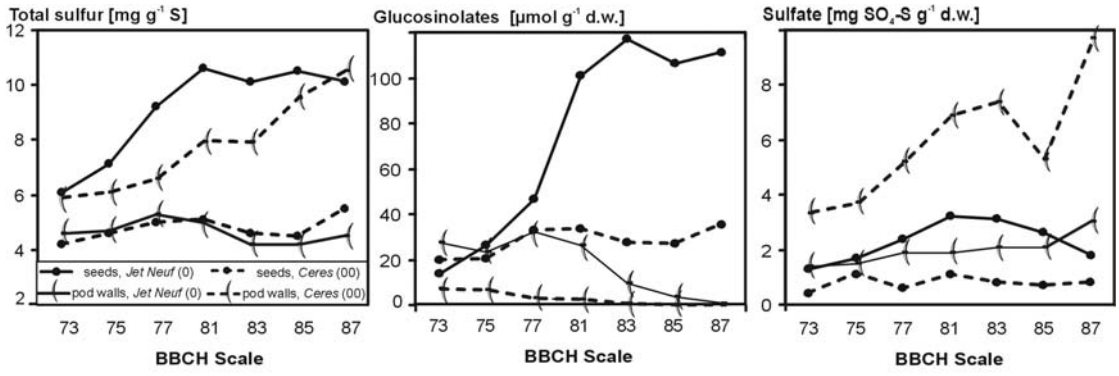

Figure 1. Changes in total sulfur, sulfate-S and total GSL content in developing seeds and pod walls of a single low and a double low oilseed rape variety (data from Bloem et al., 2007; BBCH scale according to Strauss et al., 1994).

Time-course experiments have shown that an increase in seed GSLs in developing pods was associated with a decrease in the GSL content of the pod walls what is an indication that the pod walls are the main sites of GSL biosynthesis (De March et al., 1989, Fig. 1). It was Josefsson who observed already in 1971 that the low GSL content in the Polish spring rape cultivar *Bronowski* was not caused by a reduced S uptake and he could show in radio-tracer experiments the existence of a metabolic block in GSL biosynthesis. Zhao et al. (1993) concluded from the increasing sulfate content in pod walls of double low oilseed rape varieties that a metabolic block in the GSL synthesis is responsible for the low GSL content of double low rapeseed varieties. Also Bloem et al. (2007) found increasing sulfate contents in the pod walls of double low varieties during pod development but this increase was consistent during the whole period of pod development, including the very early stages of pod development (BBCH 75-81). The fact that at the beginning of seed development the single and double low winter oilseed rape varieties behave similar in accumulating GSLs in seeds, which are most likely translocated from the pod walls into the seeds, makes it difficult to explain the phenomenon of different GSL contents in seeds of single and double low varieties. It is most likely that the transport of GSLs is no problem but that less intact GSLs are produced in double low varieties later in pod development. Moreover double low oilseed rape varieties seem to be less efficient in using S, which is indicated by lower S contents in vegetative tissues and pods in double low summer rape varieties early in pod development compared to single low varieties (Bloem et al., 2007). Most likely there is more than a single explanation for the lower GSL contents in seeds of double low varieties. The GSL content is a highly variable parameter which is not only influenced by environmental, agronomic and genetic factors, but to a great extent also by the post-harvest treatment. In general, a decrease of the GSL content after harvest is assumed when seeds are air-dried. When whole pods during pod development are, however freeze-dried and subsequently separated into pod walls and seeds in order to prevent GSL losses, Bloem et al. (2007) could show that this procedure delivered a very high variation of the GSL content, which could not be explained by seed development. It proved to be necessary to separate seeds from pod walls directly in the field to produce representative results. This procedure is particularly time-consuming during early pod development. In any case, the question remains open which apparently measured changes in S metabolites have been caused by the sampling procedure. So, an increased sulfate content in the pod walls of double low varieties might be a result of break-down from intermediary products of the GSL biosynthesis and glucosinolates, or free sulfate might have accumulated.

Application of glucosinolate research

Today most research in the field of GSLs concentrates on their use in agriculture, human nutrition and health. Next a brief overview of recent advances in this kind of GSL research are highlighted.

Biofumigation is a term which refers to the release of biocidal, fungicidal and nematicidal compounds from GSL-containing crops during green manure, crop rotation or direct application, for example as pellets, which suppress soil-borne pests and pathogens (Sarwar et al., 1998; Lazzeri et al., 2004). Research shows that the GSL-myrosinase system may provide a natural alternative for methyl bromide soil fumigation (Lazzeri et al., 2004). GSLs have no biocidal action, but their hydrolysis products, the isothiocyanates (ITCs) show biocidal action against a broad range of bacteria, fungi and nematodes in the soil (Buskov et al., 2002; Rosa et al., 1997). Because of this anti-microbial activity, GSL-containing crops have been tested as bio-fumigants. So, the incorporation of plant residues into the soil resulted in the release of biocidal ITCs (Brown and Morra, 1997). Many ITCs showed, however, a highly biocidal action in laboratory trials, while the results from field experiments often failed to show significant effects. The reason is that only a low proportion of the GSLs is transferred into ITCs (Gimsing and Kirkegaard, 2006). Calculated ITC-release efficiency accounted for 26% and 56% for high GSL rape and mustard, respectively (Gimsing and Kirkegaard, 2006). Morra and Kirkegaard (2002) could show that the greatest improvement in the use of *Brassica* biofumigants would be to develop methods to increase cell disruption and thereby maximizing GSL hydrolysis and ITC release.

The release of ITCs depends on soil pH: in soils with a pH above 5.5 ITCs will be the predominant product of GSL breakdown (Borek et al., 1996) while at lower pH other products like nitriles are released. Other soil parameters which affect the release of ITCs from GSLs are soil moisture, the organic matter and clay content as ITCs can be adsorbed to soil particles

and organic matter (Matthiessen and Shackleton, 2005).

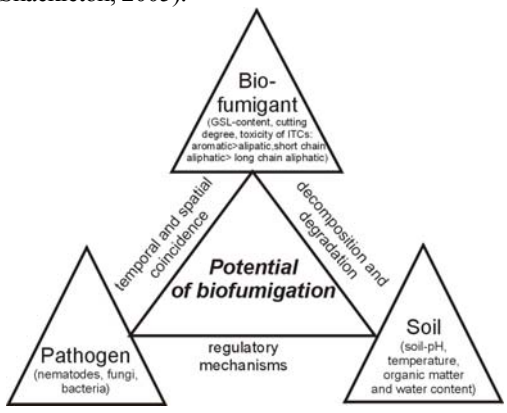

Figure 2. Factors affecting the potential of biofumigation of green manure, glucosinolate-containing crops in crop rotation and application of glucosinolate-containing products, respectively.

For a high potential of biofumigation the biofumigant needs to have a high GSL content, needs to be finely dispersed in the soil and needs to release ITCs of high toxicity. Aromatic ITCs are more toxic than aliphatic ITCs; from aliphatic ITCs those with short side-chains are much more toxic than those with longer aliphatic side chains (Manici et al., 1997; Sarwar et al. 1998). The activity of aromatic ITCs is restricted because they need to be in direct contact with the pathogen as they have a lower volatility than aliphatic ITCs (Matthiessen and Shackleton, 2005). Also important for the biofumigation potential is the coincidence of pathogen attack and the release of ITCs (Fig. 2). Gimsing and Kirkegaard (2006) could show that the concentration of GSLs and the corresponding ITCs declined rapidly during the first 4 days after incorporation of pulverized GSL-containing plant material although both could be detected in the soil for up to 8 days after incorporation. The highest levels of soil ITCs were determined in the soil immediately (30 minutes) after pulverization and incorporation of mustard with a high GSL content into the moist soil. Thus, a coincidence of ITC release and pathogen attack is not very likely if GSL-containing crops are grown in the crop rotation or as green manure. It seems to be more promising to develop a functional bio-fertilizer, with high concentrations of the most biocidal ITC-releasing GSLs and coatings which will warrant a continuous release of GSLs and ITCs and which will prevent adsorption by clay minerals or organic matter.

Pharmacological relevance of GSL-containing crops: GSLs have long been known for their anti-nutritive effects in animal nutrition. In contrast, the breakdown products of GSLs are responsible for the flavor of *Brassica* vegetables and there is increasing evidence that they act as anti-carcinogens in human aliments (Verhoeven et al., 1997; Hecht, 1999; Mithen et al., 2000). Sulforaphane, an ITC in broccoli, is an efficient inducer of Phase II enzymes which help to detoxify carcinogens (Talalay et al., 1995). Allyl ITC´s blocked the growth of cancer cells *in vitro* (Musk et al., 1995), phenylethyl ITC induced cell death (Huang et al., 1998) and sinigrin suppressed proliferation, increased apoptosis and reduced carcinogenesis in rats after treatment with a carcinogen (Smith et al., 1998). The most important reaction of ITCs is probably the modulation of the activities of Phase I and II enzymes which catalyze a variety of hydrolytic, oxidation or reduction reactions (Phase I), which products are then available for conjugation reactions (Phase II) and excretion (Mithen et al., 2000). The fact that GSLs are the principal source of anti-carcinogenic activity in *Brassica* vegetables provides a strong motive for the manipulation of GSL levels in vegetables for human consumption (Mithen et al., 2000).

Parameters influencing the GSL content are S fertilization, light intensity, temperature, growth stage of the crop, plant part, interactions with pathogens and genetic variability (Rosa et al., 1997). Just recently it could be shown that applying controlled post-harvest abiotic stress to harvested plant parts of nasturtium enhanced significantly the glucotropaeolin (GTL) content, which is an important criteria for the phytopharmaceutical value of the raw material. Drying leaves of nasturtium at 40°C in a ventilated oven increased the GTL from about 40 to > 200 % compared to freeze-dried leaf material (Bloem et al., 2006).

GSLs can be also an interesting alternative to recently banned in-feed-antibiotics in animal nutrition as ITCs may unfold anti-microbial effects. GTL from nasturtium is used against urinary tract infections of humans. Just recently a first experiment was conducted with weaning piglets to investigate the effect of different doses of GTL on feed intake, growth performance, intestinal micro-biota and benzyl-ITC concentrations in the urine. For the first time it could be shown that supplementation of animal feed with nasturtium was well-tolerated, had no influence on feed intake and growth rate of the piglets. Benzyl-ITC was found in the urine of those piglets which received nasturtium. With higher rates of supplementation also the ITC excretion increased but the total value depended very much on the amount of urine, which was excreted by the piglets (unpublished data).

Research in GSL is of prime practical relevance and recent advances in GSL research are expected to contribute substantially to food quality and sustainable agricultural production.

References

Bloem E, Haneklaus S, Schnug E (2006) Comparative effects of sulfur and nitrogen fertilization and post-harvest processing parameters on the glucotropaeolin content of *Tropaeolum majus* L. J. Sci. Food Agric. (in press).

Bloem E, Haneklaus S, Schnug E (2007) Changes in the glucosinolate and total sulphur content of developing seeds and pods of single low and double low

summer and winter oilseed rape varieties (*Brassica napus* L.) J Plant Nutr. (in prep).
Borek V, Morra MJ, McCaffrey JP (1996) Myrosinase activity in soil extracts. Soil Sci. Soc. Am. J. 60:1792-1797.
Brown PD, Morra MJ (1997) Control of soil-borne plant pests using glucosinolate-containing plants. Adv. Agron. 61: 167-231.
Buskov S, Serra B, Rosa E, Sorensen H, Sorensen JC (2002) Effects of intact glucosinolates and products produced from glucosinolates in myrosinase-catalyzed hydrolysis on the potato cyst nematode (*Globodera rostochiensis* Cv. Woll). J. Agric. Food Chem. 50: 690-695.
De March G, McGregor DI, Séguin-Shwartz G (1989) Glucosinolate content of maturing pods and seeds of high and low glucosinolates summer rape. Can. J. Plant Sci. 69: 929-932.
Fieldsend J, Milford GFJ (1994) Changes in glucosinolates during crop development in single- and double-low genotypes of winter oilseed rape (*Brassica napus*): I. Production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within the crop. Ann. Appl. Biol. 124: 531-542.
Gimsing al., Kirkegaard JA (2006) Glucosinolate and isothiocyanate concentration in soils following incorporation of *Brassica* biofumigants. Soil Biol. Biochem. 38: 2255-2264.
Hecht SS (1999) Chemoprevention in cancer by isothiocyanates, modifiers of carcinogen metabolism. J. Nutr. 129: 768S-774S.
Huang C, Ma WY, Li J, Hecht SS, Don Z (1998) Essential role of p53 in phenylethyl isothiocyanate-induced apoptosis. Cancer Res. 58: 4102-4106.
Lazzeri L, Leoni O, Manici LM (2004) Biocidal plant dried pellets for biofumigation. Indust. Crops Prod. 20: 59-65.
Josefsson E (1971) Studies of the biochemical background to differences in glucosinolates content in *Brassica napus* L. I. Glucosinolate content in relation to general chemical composition. Physiol. Plant. 24: 150-159.
Manici LM, Lazzeri L, Palmieri S (1997) In vitro fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. J. Agric. Food Chem. 45, 2768-2773.
Matthiessen JN, Shackleton MA (2005) Biofumigation: environmental impacts on the biological activity of divers pure and plant derived isothiocyanates. Pest Managem. Sci. 61 (11): 1043-1051.
Mithen RF, Dekker M, Verkerk R, Rabot S, Johnson IT (2000) The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J. Sci. Food Agric. 80: 967-984.
Moreno DA, Carvajal M, Lopez-Berenguer C, Garcia-Viguera C (2006) Chemical and biological characterisation of nutraceutical compounds of broccoli. J. Pharmaceut. Biomed. Anal. 41: 1508-1522.
Morra MJ, Kirkegaard JA (2002) Isothiocyanate release from soil-incorporated *Brassica* tissues. Soil Biol. Biochem. 34: 1683-1690
Musk SR, Stephenson P, Smith TK,Stening P, Fyfe D, Johnson IT (1995) Selective toxicity of compounds naturally present in food toward the transformed phenotype of human colorectal cell line HT29. Nutr. Cancer 24: 289-298.
Rosa E, Heaney RK, Fenwick GR, Portas CAM (1997) Glucosinolates in crop plants. Hortic. Rev. 19: 99-215.
Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier JM (1998) Biofumigation potential of brassicas - III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. Plant and Soil 201: 103-112.
Schnug E (1990) Glucosinolates – fundamental, environmental and agricultural aspects. In: Sulfur nutrition and sulfur assimilation in higher plants, (Rennenberg et al. eds.), The Hague: SPB Acad. Publ. pp. 97-106.
Schnug E (1993) Physiological functions and environmental relevance of sulfur-containing secondary metabolites. In: Sulfur nutrition and assimilation in higher plants. (De Kok et al. eds.) The Hague: SPB Acad. Publ. pp.179-192.
Smith TK, Lund EK, Johnson IT (1998) Inhibition of dimethylhydrazine-induced aberrant crypt foci and induction of apoptosis in rat colon following oral administration of the glucosinolate sinigrin. Carcinogenesis 19: 267-273.
Strauss R, Bleiholder H, van den Boom T, Buhr L, Hack H, Hess M, Klose R, Meier U, Weber E (1994) Einheitliche Codierung der phaenologischen Entwicklungsstadien mono- und dikotyler Pflanzen. Ciba-Geigy AG, Basel, 56 pp.
Talalay P, Fahey JW, Holtzclaw WD, Prestera T, Zhang Y (1995) Chemoprotection against cancer by phase 2 enzyme induction. Toxicol. Lett. 82/83: 173-179.
Verhoeven DT, Verhagen H, Goldbohm RA, van den Brandt PA, van Poppel GA (1997) Review of mechanisms underlying anticarcinogenicity by brassica vegetables. Chem. Biol. Interact. 103: 79-129.
Zhao FJ, Evans EJ, Bilsborrow PE,Syers JK (1993) Sulphur uptake and distribution in double and single low varieties of oilseed rape (*Brassica napus* L.). Plant and Soil 150: 69-76.

Quality of canola (*Brassica napus* L.) varieties in Western Canada: Evaluation of variability due to genetic, year and environmental conditions using data from Canadian Grain Commission Harvest Surveys and from Environment Canada meteorological stations

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Abstract

Quality data (oil content, crude protein content (as oil-free protein and seed protein), the sum of oil and protein content and chlorophyll content) from Canadian Grain Commission harvest surveys coupled with meteorological data from Environment Canada (monthly minimum and maximum temperatures and monthly total precipitation by provincial crop district from 1992 to 2003) were used to evaluate quality factors for varieties of *B. napus* canola grown in Canada between 1992 and 2005. The analysis was restricted to the most popular varieties that appeared in the survey at least 3 years and for which there were at least 20 data points per year. Analysis of annual parameter means and overall variety ranks showed significant increases in protein content and oil plus protein content were noted in *B. napus* canola over the period but although oil content increased, the increase was not statistically significant. Chlorophyll content did not increase in harvest survey data but has increased in exports over the period. Statistically significant differences were found between varieties for all of the above parameters and a strong variety by location (province) effect. Sample variance within a variety was used as a rough measure of environmental sensitivity. There were significant genetic differences in the sample variance between varieties and in many cases this was consistent across years and locations. Significant correlations were noted for meteorological parameters for oil content (low temperatures in June), protein content (maximum temperatures in June and July), oil plus protein (minimum temperatures in August), chlorophyll (minimum temperatures in September) and there were significant variety differences in the responses (slopes) to these parameters. This suggests that it should be possible to design breeding strategies to develop varieties with greater or lesser sensitivity to environmental fluctuations.

Key words: quality, environment, variety, oil, protein, chlorophyll, temperature, precipitation, variability

Introduction

In order to be recommended for registration for growing in Western Canada, varieties of canola are evaluated for quality according to the rules and direction of the Western Canada Canola/Rapeseed Recommending Committee (WCC/RRC). These rules establish minimum requirements and incentives in the case of oil content and protein content for the committee's recommendation of new varieties for registration in Canada. Concerns about the performance of new canola varieties have been expressed but most of these might be put down to the recent changes in species balance (Daun, J. K. and DeClercq, D. R., 98) (Daun, J. K., 2003). At the same time the Canola Council of Canada (CCC) has undertaken a review of canola quality (Hickling, D., 2005) identifying chlorophyll, oil, protein and saturated fatty acids as ongoing issues. Furthermore, a review of environmental and agronomic factors (Goodwin, 2004) concluded that recent variations in oil content, chlorophyll and oil profile are due mostly to meteorological conditions. The study did not find a clear relationship between new genetic innovations and oil content.

Ideally, data from controlled plot studies such as variety registration trials would be most valuable in this type of study. In the Canadian situation, however, varieties require only one year of public testing before registration and the turnover of varieties, including check varieties, in these trials is very rapid. Another source of information is the Canadian Grain Commission (CGC) quality surveys of newly harvested Canadian rapeseed and canola crops (Declercq, D. R. and Daun, J. K., 2005). Since 1994, reliable data on oil and protein content, chlorophyll and more recently saturated fatty acids has been generated by NIR analysis of the individual samples in these surveys.

The present study was undertaken in order to provide a more in depth study of the performance, in terms of quality of the major canola varieties grown in Western Canada over the past 12 years. The study utilizes data from CGC surveys to evaluate differences between the major B. napus canola varieties in terms of oil content, protein content (of the meal), saturated fatty acids and, if possible, chlorophyll content. In addition, the study attempts to determine whether there are any differences between the varieties response to growing location or environmental factors for these parameters. (Daun 2006)

Materials and Methods

Statistical Methods Statistical procedures used were from either SAS version 9.1 for windows SAS Institute Inc., Cary, NC, USA., Origin version 7.5 Originlab Corporation, Northhampton MA, USA, Graphpad Instat version 3.05 GraphPad Software, San Diego CA, USA or Microsoft Excel 2002 Microsoft Corporation, Seattle, WA, USA. The following procedures were used.

Correlation: Pearson correlation coefficients were determined for the relationship between quality parameters (averaged by variety by provincial crop district) and meteorological data. The number of samples in each crop district was used as a weighting factor.

Analysis of Variance: Both simple and factorial ANOVA's were run using SAS. Type III sums of squares were used. Means tests were carried out using either the Bonferroni method, which accepts unequal sample sizes or by the Duncan's test. Repetitions found that there were no significant differences in the results by the two different means tests but the Bonferroni results were used where there were significant differences in the number of groups.

Regressions: Quality factors averaged by crop district were regressed with meteorological data using simple linear regression where correlations were significant and consistent in sign across the varieties. The number of samples per crop district was used as the frequency. Slopes for the regression lines constructed were compared using the Bonferroni method on Graphpad Instat. Regressions between quality factors and meteorological factors and location (latitude and longitude averages for crop district) were also constructed using forward selection with $\alpha = 0.05$. Partial correlations (expressed as %) were compiled and gave an indication of the factors that might be important for future study.

Comparison of Coefficiants of Variation: The Brown-Forsythe method of comparing variances was used to construct groups of equal variances by repeated analysis of variance using the Origin software. While this may not be a recognized statistical process, it does provide some interesting information. The Brown-Forsythe test is more robust than the alternative Levene test but both are questionable where samples have large differences in numbers as was the case in the data studied.

Harvest Survey Database of Canola Quality. Each year since 1956 the Canadian Grain Commission has surveyed the quality of Canadian rapeseed and canola by obtaining and testing samples of farm deliveries or farm harvested crop. The results of these have been published as crop bulletins and related reports to the Canadian canola and rapeseed industry. Databases of information gathered from these surveys also have been established and information in these was used in the current study.

Database of Environmental Information. A database of environmental information containing average values for monthly minimum and maximum temperature and precipitation by provincial crop district for the years 1971 to 2002 was prepared from Environment Canada data by the National AgriClimate Group of Agriculture Canada's Prairie Farm Rehabilitation Administration (courtesy Mr. Trevor Hadwen, AAFC-PFRA, Regina).

Results and Discussion

Oil Content. While oil content of Canadian canola has increased over the long term (Fig. 1), there was little evidence of an increase over the past 14 years, either in the overall crop or in varieties (Fig. 2). There was a 3 percent difference in the mean value of oil content between the variety with the highest and the variety with the lowest oil content and there were significant differences between varieties both in their mean values and in the variation about those mean values (Figure 3).

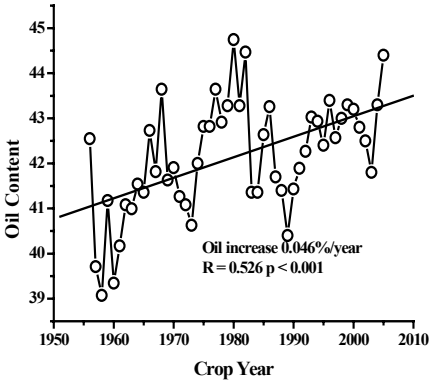

Figure 1. Oil content of Canadian Canola from CGC Harvest Surveys

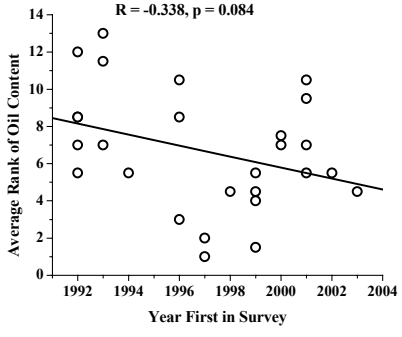

Figure 2. Rank of oil content for all varieties tested between 1994 and 2006 plotted against the first year the variety appeared in the survey.

Some varieties showed low variability in oil content in each year studied (Fig. 4). Location, as expressed by province also had a significant effect with samples from Alberta having the highest oil contents and those Manitoba the lowest. Some varieties showed a greater stability to location than others.

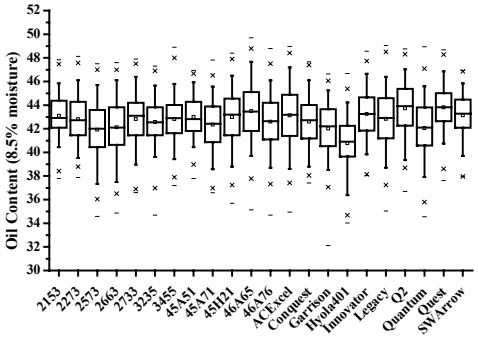

Figure 3. Mean and ranges of oil contents for varieties of Canadian Canola, 1994 to 2006.

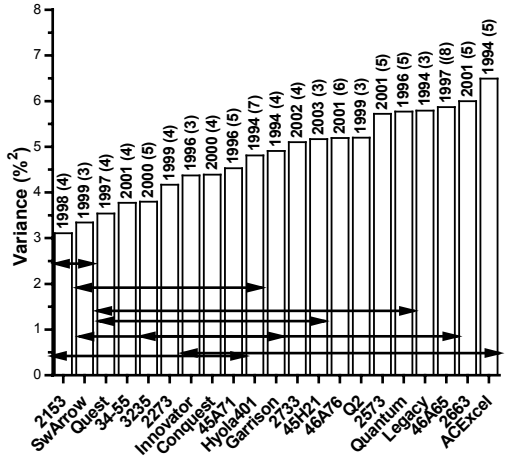

Figure 4. Comparison of variances for oil content between major varieties in the harvest survey between 1994 and 2005 showing only varieties with more than 20 samples per year for at least 3 years. Labels show the year first in the survey and the number or years in the survey (not necessarily contiguous). Arrows show groups of variances that are not significantly different according to the Brown-Forsythe test.

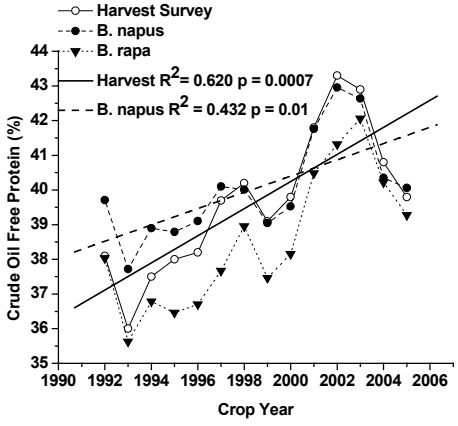

Figure 5. Average crude protein contents of oil-free Canadian canola and rapeseed from Canadian Grain Commission Harvest Surveys, 1992 to 2005. showing differences due to species makeup.

Table 1. Comparison of June minimum temperature effect on oil content between varieties. The regressions were weighted by the number of data points (variety oil contents) per crop district.

Variety	Data Points	Slope[1]	Std. Dev.	Group	R^2
AC Excel	82	-0.813	0.127	A	0.479
Garrison	63	-0.682	0.111	B	0.378
Legacy	57	-0.679	0.117	B	0.654
Quantum	94	-0.409	0.106	C	0.163
Hyola401	92	-0.391	0.087	C	0.300
2273	52	-0.260[2]	0.155	D	0.080
46A65	101	-0.252	0.114	D	0.155
Innovator	56	-0.239	0.105	D	0.088

[1] change in oil with each ºC increase in Minimum June Temperature
[2] not significantly different from 0

Minimum temperatures, particularly in June but also in May through August, had an effect on oil content and different varieties responded to different degrees to this effect (Table 1).

Crude Protein Content. Oil-free protein content remained relatively constant the long term but over the past 12 years it increased dramatically both in part due to the shift from a mixture of *B. rapa* and *B. napus* to almost complete *B. napus* and also due to a significant increase in the crude oil free protein in *B. napus* varieties (Figure 5).

As with oil content, there were significant differences between variety means and variances and some varieties showed consistency variance in each of the year's studied. Samples from Manitoba had the highest oil free protein content and those from Saskatchewan the lowest. Maximum temperatures in June and July played a significant role in determining crude oil free protein content and there were variety differences in the magnitude of that effect (Table 2).

Table 2. Comparison of July maximum temperature effect on oil free protein content between varieties. The regressions were weighted by the number of data points (variety oil contents) per crop district

Variety	Data Points	Slope[1]	Std. Dev.	R^2	Group[2]
v46A65	101	0.049	0.015	0.037	A
vQuest	80	0.058	0.009	0.079	B
v46A76	60	0.067	0.016	0.061	C
vQuantum	94	0.069	0.008	0.086	C
vConquest	47	0.082	0.019	0.100	D
v3235	39	0.090	0.022	0.147	E
vInnovator	56	0.092	0.017	0.099	E
v2273	52	0.103	0.028	0.097	F
vGarrison	63	0.103	0.018	0.127	F
v3455	38	0.105	0.015	0.310	F
v45A71	79	0.110	0.012	0.202	F
vQ2	38	0.121	0.032	0.117	G
vACExcel	82	0.128	0.015	0.138	GH
vLegacy	57	0.130	0.017	0.172	H
vSwArrow	48	0.139	0.026	0.182	H
v2153	54	0.151	0.027	0.230	I

[1] change in oil free protein with each ºC increase in Maximum July Temperature

[2] The Bonferroni test used in Table 7 was considered to be too conservative and the Student-Neuman-Keul test was used instead. Note that the standard deviations were found to be significantly different.

Sum of Oil and Protein Content. The sum of oil and protein content has increased over the long term, mostly due to increases in oil content but more recently due to the effect of increased meal protein content coupled with a steady (or slightly increasing) oil content. As with other parameters there were significant variety differences in means and variability about the means although the year to year consistency of the response by variety was not noted. Samples from Alberta had the highest oil plus protein content while those from Saskatchewan had the lowest. August minimum temperatures had a significant role in establishing oil plus protein content and there were variety differences in the magnitude of the effect (Table 3)

Table 3. Comparison of August minimum temperature effect on oil plus protein content between varieties. The regressions were weighted by the number of data points (variety oil contents) per crop district

Variety	Data Points	Slope[1]	Std. Dev.	R^2	Group
Legacy	57	-0.889	0.279	0.156	A
v34-55	38	-0.857	0.342	0.148	A
Quantum	94	-0.568	0.183	0.094	B
Conquest	47	-0.542	0.234	0.107	B
v2273	52	-0.434	0.176	0.107	B
v46A76	60	-0.431	0.198	0.075	B
Ac Excel	82	-0.294	0.111	0.081	C

[1] Change in Oil Pluse Protein Content (%) with each ºC increase in Minimum August Temperature

Table 4.. Comparison of September minimum temperature effect on chlorophyll content between varieties. The regressions were weighted by the number of data points (variety oil contents) per crop district.

Variety	Data Points	Slope[1]	Std. Dev.	R^2	Group
Quest	80	-0.133	0.043	0.110	A
2153	54	-0.132	0.040	0.171	AB
Garrison	63	-0.113	0.028	0.215	B
ACExcel	82	-0.094	0.023	0.171	C
45A71	79	-0.085	0.025	0.127	DD
2273	52	-0.062	0.026	0.103	E
46A65	101	-0.053	0.025	0.045	E

[1] Change in Chlorophyll Content (mg/kg) with each ºC increase in Minimum September Temperature

Chlorophyll Content. There was no long term trend in chlorophyll content as this factor is highly weather dependent. This weather dependency should be kept in mind when considering other observations and conclusions. Chlorophyll contents of top grade exports have increased over the past 10 years, mostly due to the lower amounts of the low chlorophyll *B. rapa* varieties in the crop. Significant differences in means and variability of chlorophyll were noted between varieties but there was no trend towards the development of lower chlorophyll varieties. Samples from Alberta and Saskatchewan had more chlorophyll than samples from Manitoba. Minimum temperatures in September played a significant role in establishing chlorophyll content and there were variety differences in the effect (Table 4).

Acknowledgements

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References

Daun, J.K. How green is green? Long-term relationships between green seeds and chlorophyll in canola grading. *J. Amer. Oil Chem. Soc.* **2003**, *80*, 119-122.

Daun, J. K. *Quality of canola (Brassica napus L.) varieties in Western Canada: Evaluation of variability due to genetic, year and environmental conditions using data from Canadian Grain Commission Harvest Surveys and from Environment Canada meteorological stations*. Canola Council of Canada, Winnipeg, 2006.

Daun, J.K.; DeClercq, D.R. Saturated Fatty Acids in Canadian Canola. Bull. GCIRC 1998, 15, 27-29

Declercq, D.R.; Daun, J.K. Canadian Canola Quality Parameters. *Bull. GCIRC* **2005**, *22*, http://195.101.239.21/publications/B22/canadiancanola%20quality.htm.

Goodwin, M. *A review of the impact of environment and agronomic practices on quality of Canadian canola oil*; Canola Council of Canada: Winnipeg, 2004.

Hickling, D. *Canola Quality Review*; Canola Council of Canada: Winnipeg, 2005.

A preliminary study on glucosinolates heterosis in leaf of hybrids in *Brassica napus* L.

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Abstract

Brassica napus L. is widely grown in Yangzi River valley of China as a major edible oilseed. Because of the unpleasant smell and toxicity from glucosinolates degradation products, genetic improvement has been made to decrease glucosinolates concentration in seed by breeding. However, it was also found that leaf glucosinolates concentration and profiles were closely related to plant diseases resistance and the taste of shoot as vegetable. And anticancer effects from degradation products of indolyl and other glucosinolates were also reported. Recently, new varieties of *Brassica napus* L. used both for vegetable and oilseeds were released and popularized in the middle and lower valley of Yangzi River. Rapeseed with high glucosinolate content in leaves and low content in seeds were considered as ideal for both seed quality and plant diseases resistance. Therefore leaf glucosinolates call for more and more attention. In this paper the variation of leaf glucosinolates content in all growth periods and the heterosis of glucosinolates in leaf were studied with hybrids and their parents as well as the double high material by high performance liquid chromatography (HPLC). The results showed that Leaf glucosinolate content in double-high material Zhongyou 821 decreased from rosette to blooming stage, while in double-low material leaf glucosinolate increased from overwintering to budding period, decreased at stem elongation stage and rose again at early blooming stage. Leaf glucosinolate contents and profiles varied significantly in double-high and double-low rapeseeds although aliphatic glucosinolates were the dominant ones in both leaves. All crosses presented mainly negative heterosis over both parents. The heterosis of leaf glucosinolates of F_1 and F_2 hybrids were significant and negative in stem elongation and floral period, suggesting that it would be difficult to increase leaf glucosinolates in these periods for high disease resistance and it might be the best time for harvesting shoots as vegetable.

Key words: *Brassica napus*, leaves, growth stage, glucosinolates, heterosis

Introduction

Rapeseed in which *Brassica napus* accounts for more than 90 percent is the most important oilseed cultivated in China as the main edible vegetable oil source and animal feedstuff. The presence of glucosinolates in rapeseed influences the quality of meal obtained after crushing the seeds as break-down products of the glucosinolates are goitrogenic which results in depressed growth of the animals fed with meals containing high level glucosinolates. However, it has also been shown that there are positive effects from glucosinolates degradation products such as the increase of diseases resistance. Indolyl glucosinolates degradation products were reported to increase the mouse immunity. And rapeseed plant organs inoculated with high Indolyl glucosinolates were found to be more resistant to pests. Milford showed that the degradation products of glucosinolates could prevent rapeseed plant from mechanical damage and pests. Seed glucosinolates genetics has been widely studied and the concentration especially aliphatic ones in seed has been significantly reduced by double low breeding program. In this study, glucosinolates profiles, concentration and heterosis in growth stages of leaf in *Brassica napus* were investigated by high performance liquid chromatography.

Materials and methods

Rapeseed leaves materials from *Brassica napus* 1008,5899,6098,M_1,R_1,R_6, Zhongyou 821 and F_1, F_2 populations were planted in the Farm of Oil Crops Research Institute of Chinese Academy of Agricultural Sciences. Rapeseed leaves were cut and frozen with liquid nitrogen at resetting, overwintering, budding, stem elongation, early blooming and blooming stages. Leaves samples were stored in freezer at -45°C and dried by vacuum freezing at -45°C.

Leaves samples were grinded with microgrinder and extracted with 70% methanol (70%,v/v solution) by ultrasonic for 18 min after 1min water bath at 75°C for inactivation of myrosinase. Sample was transferred to mini ion-exchange column with sulfatase and kept for 20 hours. After glucosinolate was eluted, samples were analyzed by high performance liquid chromatography. Waters M32 HPLC system with 510 pumps, 470auto-injector, connected to a 2487 ultraviolet detector was used. Glucosinolates were separated with a Novapak C_{18} column at 30°Cand detected at the wavelength of 229 nm.

Results and discussions

Leaves glucosinolates profiles at different growth stages

Concentration and components change of glucosinolates with growth stages of *B.napus* leaves was showed in Fig. 1 and Table 1. Glucosinolates content varied significantly among the materials and growth stages. Glucosinolates content in double high material Zhongyou 821 decreased from rosetting to blooming stage with the maximum content of 33.15 μmol·g^{-1}, while in double-low material leaf glucosinolate increased from overwintering to budding period, decreased at stem elongation stage and rose again at early blooming stage. The maximum value of M_1, 1008, 6098 × 1008 and 6098 were 9.40, 7.11, 6.35 and 7.51μmol·g^{-1}, respectively.

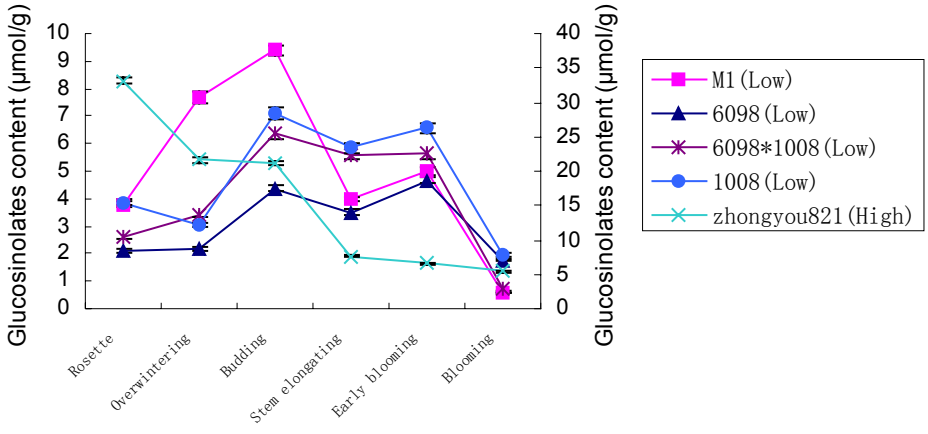

Table 1 Concentration of glucosinolates at different grow stages of *B.napus* leaves (μmol·g^{-1})

Material	Total	A	B	C	D	E	F	G	H	I	J	K
1008	5.45	1.97	0.10	0.23	0.19	0.52	0.27	0.81	0.67	0.59	0.13	0.09
5899	4.97	0.63	0.97	0.03	0.09	0.98	0.17	0.17	0.39	2.07	0.10	0.12
6098	4.71	0.77	0.26	0.44	0.48	1.35	0.26	0.44	0.31	0.79	0.13	0.13
M_1	5.20	0.46	0.28	0.12	0.12	0.26	0.02	-	0.09	3.64	0.30	0.08
6098 × 1008	4.52	1.09	0.15	0.33	0.33	0.82	0.32	0.38	0.44	0.73	0.16	0.14
6098 × M_1	3.02	0.71	0.16	0.24	0.12	0.53	0.08	0.17	0.15	0.84	0.12	0.07
5899 × 1008	3.10	0.73	-	-	0.20	0.43	0.23	0.20	0.46	0.65	0.09	0.16
R_1	0.85	0.15	0.23	0.03	0.06	0.05	0.02	-	0.07	0.50	0.04	0.08
R_6	4.18	1.64	-	0.10	0.10	0.71	0.38	-	0.61	0.50	0.07	0.12
821	17.53	8.94	0.49	0.07	0.75	4.17	1.17	1.06	2.17	1.80	0.23	0.32

A.Desulfoprogoitrin; B.Desulfoglucoraphanin; C. Desulfogluconapoleiferin; D. Desulfoglucoalyssin; E. Desulfogluconapin; F. Desulfo-4-hydroxyglucobrassicin; G. Desulfoglucobrassicanapin; H. Desulfoglucobrassicin; I. Desulfogluconasturtin; J. Desulfo-4-methoxyglucobrassicin; K. Desulfo-1-methoxyglucobrassicin

Aliphatic, aromatic and indolyl glucosinolates in *B.napus* leaves

The profile of aliphatic, aromatic and indolyl content in *B.napus* leave was showed in Fig.2. The content of aliphatic glucosinolates was from 0.51 to 14.42 μmol·g^{-1}, which was the dominant glucosinolates accounting for 23.05 to 68.13 percent of the total content. The aromatic gluocosinolates content was from 0.50 to 3.64 μmol·g^{-1}, accounting for 8.51~68.00 percent of the total content. The concentration of indolyl glucosinolates was from 0.20 to 4.94 μmol·g^{-} accounting for 8.94%~36.26 percent of the total glucosinolates content.

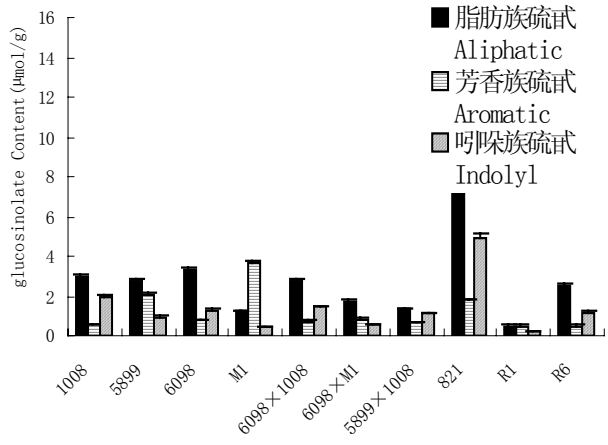

Fig. 2 Profile of aliphatic, aromatic and indolyl glucosinolates content in *B.napus* leaves

Table 2 showed correlations among all three types of glucosinolates. The correlations between aliphatic,indolyl and all glucosinolates components were significant with coefficient 0.98 and 0.95. The correlation between aliphatic and aromatic ones was also significant with correlation coefficient 0.97.

Table 2 Correlations between aliphatic, aromatic, indolyl and total glucosinolates content in *B.napus* leaves

Item	All individuals	Aliphatic	Aromotic
Aliphatic	0.98**		
Aromatic	0.32	0.16	
Indolyl	0.95**	0.97**	0.05

*Significant (P≤0.01)

**Very significant(P≤0.05)

Glucosinolates Heterosis in Leaf of Hybrids in Brassica napus *L.*

Glucosinolates content differences in leaves of *B.napus* between double low and double high materials were mainly from aliphatic glucosinolates variation. Progoitrin, gluconapin,,4-hydroxyglucobrassicin, glucobrassicanapin and glucobrassicin are the main components of glucosinolates in leaves. It would be beneficial to plant diseases resistance when the parents material selected with high concentration of those glucosinolates above were used for crossing. The heterosis of leaf glucosinolates were significant and negative in stem elongation and floral period, suggesting that it is difficult to increase leaf glucosinolates in these periods for high disease resistance and it would be the best time for harvesting shoots as vegetable.

References

Wang H Z. Rapeseed industry in China after joining in WTO. Chinese Journal of Oil Crop Sciences, 2002, 24(2): 82-86. (in Chinese)

Opalka M, Dusza L, Koziorowski M, Staszkiewicz J, Lipiński K, Tywończuk J. Effect of long-term feeding with graded levels of low glucosinolate rapeseed meal on endocrine status of gilts and their piglets. Livestock Production Science, 2001, 69: 233-243.

Tan X L, Tian J H, Li D R. Study on seed glucosinolate compositions differences between parents of *Brassica juncea* and *Brassica napus* and their progenies. *Acta Botanica Boreali-Occidentalia Sinica*, 1997, 17: 382-386. (in Chinese)

Wen B L, Li P W, Li H S, Zhao L, Yu P, Zou J Q, Zhang W, Ding X X, Yang M, Wang X F, Wu Y. Studies on antitumor effect of indolyl glucosinolate degradation products from rape on mice transplanted S_{180} cell. *Chinese Traditional and Herbal Drugs*, 2002, 33: 331-333. (in Chinese)

Li Y, Kiddle G, Bennett R N, Wallsgrove R M. Local and systemic changes in glucosinolates in Chinese and European cultivars of oilseed rape (*Brassica napus* L.) after inoculation with *Sclerotinia sclerotiorun* (stem rot). *Annals of Applied Biology*, 1999, 134: 45-58.

Bhardwaj H L, Hamama A A. Accumulation of glucosinolate, oil, and erucic acid in developing *Brassica* seeds. *Industrial Crops and Products*, 2003, 17(1):47-51.

Agerbirk N, Ørgaard M, Nielsen J K. Glucosinolates, flea beetle resistance, and leaf pubescence as taxonomic characters in the genus *Barbarea (Brassicaceae)*. *Phytochemistry*, 2003, 63: 69-80.

Li P W, Michaelsen S, Møller P and Sørensen H. Micellar electrokinetic capillary chromatography as a fast, cheap, and efficient HPCE method for separation and quantification of intact and desulfo glucosinolates. *GCIRC Bulletin*, 1994, 174: 155-160.

Zhao J Y, Zhang D Q. Study of glucosinolate components between nutritive organs and reproductive organs in *Brassica napus*. *Zhejiang Agricultural Sciences*, 1990, 6: 257-260. (in Chinese)

A survey plan for a better management of quality from plate to field

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Abstract

Food safety became a priority for consumers and the European regulation requires, since the beginning of 2006, the control of food safety risks all along the chain from oilseeds crop production to oil and meal.

A survey plan has been set up since 2003 in order to improve knowledge about the possible contamination level in rapeseed products and about the main critical points located in the seed production, storage and processing. This network, managed by CETIOM, in collaboration with ONIDOL (National agency for oilseeds development) and ITERG (Technical research centre for fats and oils industry), include professional organizations representing the oilseed foodchain : seed production, storage, crushing, refining and animal feeding. The main contaminants of interest are pesticides residues (which can occur in crude oils), heavy metals, *salmonellas* and mycotoxins. The companies which accept to join the survey plan send to CETIOM their contaminants data (obtained by their own sampling and analytical methods) and contribute in this way to build the databases which are furthermore completed by the results of annual contaminants analyses in seeds, oils and meals, these ones being managed by CETIOM, ONIDOL and ITERG. In the same time, 25 laboratories joined a working group for developing reliable analytical methods for pesticides residues in seeds and oil.

The survey plan has now been operating for 2 years and 20 companies are actually involved for the implementation of three databases on seeds, oils, and meals.

The survey plan of oilseeds is an efficient tool for proposing responses based on consensus at the time of critical safety situations, setting realistic maximum limits in French and European regulations, focusing research on well identified problems, developing traceability methodology and the application of HACCP all along the rapeseed production and processing chain.

Key words : rapeseed, contaminants, food safety, survey plan

Introduction

Faced with the confidence crisis of the consumers, the requirements about food safety are strengthening. At any level of the food and feed oilseed chain, the operators have to adapt to the new regulatory context. The White Paper published in January 2000, by the European Commission, set the agenda of the measures to be taken. It reminds that food operators, feed manufacturers and farmers have the primary responsibility for food safety. It points out that limits of contaminants must be set and controlled : pesticides residues, contamination by mycotoxins, heavy metals, polyaromatic compounds (PAHs) etc). From the upstream to the downstream, the setting up of analytical procedures and risk assessment becomes a necessity. From 2001, some pesticides residues were related by French crushing industry in crude oils, mainly rapeseed and sunflower. Although these residues are eliminated during the different steps of refining, it was important to know the origin of these contaminations to minimize the risks through good management practices and education. A study led between 2003 and 2004 showed that the main contaminants were organophosphate compounds (dichlorvos, chlorpyriphos-methyl, pyrimiphos-methyl, malathion) and pyrethrinoids (deltamethrin) which are insecticides traditionally used for cereal grain treatments during storage. The French regulation does not authorize these insecticides for the treatment of stored oilseeds which have however an affinity for liposoluble chemicals and can pick these up from storages which have previously been treated or used to store treated cereal grains. Otherwise, some *salmonellas* contaminations may occur in rapeseed meal ; moreover, questions are asked about heavy metals. In order to ensure a complete exploration of the contamination throughout the rapeseed chain, from seeds to oil and meal, the French oilseeds network decided to set up an operational survey plan of rapeseed contaminants.

Materials and methods

The survey plan is built with the following objectives :

- to identify the main critical points, from seeds production to oil and meal, as possible sources of contamination and to check the contaminants to be considered,
- to set up a database of these contaminants in seeds (rapeseed and other oilseeds), oil and meal,
- to compare the data with the available regulatory maximum limits,
- to develop and propose suitable analytical methods for determination the pesticides contents in seeds,
- to propose research actions on identified gaps,
- to contribute to set realistic regulatory limits,
- to be able to give answers based on consensus in case of crisis in the rapeseed field.

Structure and running of the survey plan (figure 1)

Firms belonging to the different professional organizations of rapeseed network (storage, crushing, oil refining and feedstuffs) are invited to join the survey plan. The management is made by Cetiom in collaboration with a working group composed of the different professional organizations with the support of scientists. The firms which accept to join the survey plan send to CETIOM their contaminants data (obtained by their own sampling and analytical methods) and contribute in this way to build the database.

This database is completed by the results of yearly contaminants analyses made in seeds, oils and meals, these ones being managed by CETIOM, ONIDOL (National agency for oilseeds development) for seeds and meals, and ITERG (Technical research center for fat and oil industry) for oil.

CETIOM (for seeds and meals) and ITERG (for oil) synthesize the results and send to each partner an annual confidential report.

Adjustment of reliable methods for analysis of pesticides in seeds and oil

Few methods are standardized at international level (ISO) for sampling and analysis of contaminants and pesticides residues and methods used at national level are often home-made methods developed in industrial or official laboratories. Some official maximum limits (MRLs) are set for contaminants and residues in oils. A network composed of 25 laboratories and managed by CETIOM and ITERG is developing suitable analytical methods for measuring pesticides, mainly insecticides, in seeds and oil. Sixteen compounds (organophosphorous organochlorous and pyrethrinoids) are actually studied. The aim is to propose accurate and standardized methods at international level (ISO) and to increase in this way the reliability of the survey plan.

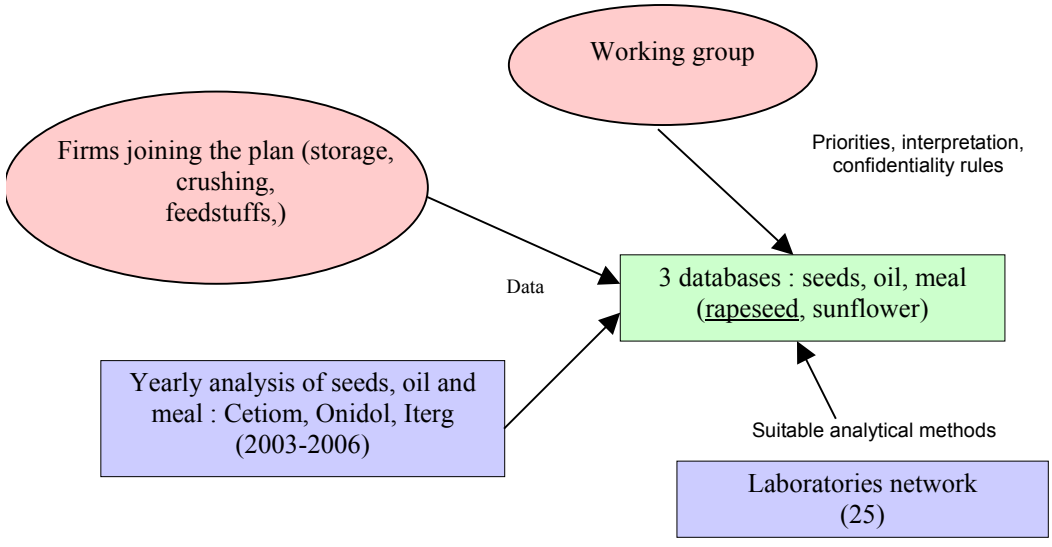

Figure 1 : Structure and running of the survey plan

Results

Main contaminants and critical points

The main potential contaminants are heavy metals (transfer from soil to plant), mycotoxins (developing during pre-harvest or post-harvest period), *salmonellas* (occurring during meal storage) and pesticides residues (from treatments on crops or accidental insecticides contaminations during seed storage. The contaminants can be concentrated, relocated or removed all along the chain from rapeseed crop to oil and meal (figure 2). For example, pesticides residues are eliminated during refining ; heavy metals are concentrated in meals.

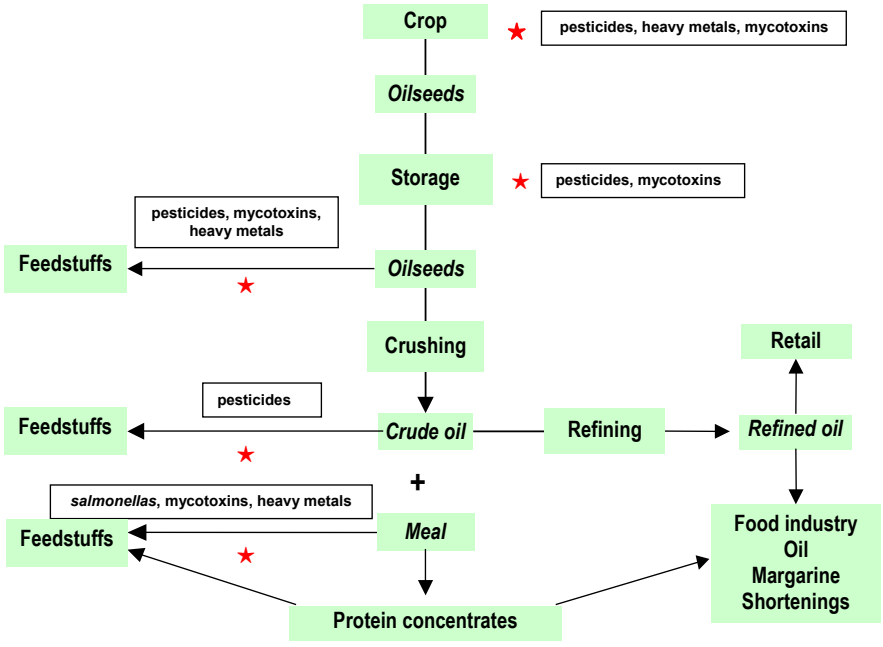

Figure 2 : From crop to oil and meal : the main critical points and potential contaminants

The survey plan

The survey plan is running for two years and 20 companies have signed an agreement (8 in seed storage area, 7 for crushing and refining, 5 feedstuffs companies). A common software to grains (French cereals survey plan managed by IRTAC) and oilseeds ("QUALISURVEY") is now operating and 2 reports have been produced in 2005 (harvest 2004) and in 2006 (harvest 2005).

To day, 2 800 data (seeds and meal) and 3 500 data (oil) - rapeseed, sunflower and soybean - have been supplied.

Yearly analysis of contaminants

The sanitary quality survey began in 2003 for a period of four years. Concerning the rapeseed, 25 samples of seeds, 25 samples of meal and 25 samples of oil were analysed in 2003 (corresponding to the harvest 2002) for the following contaminants (tableau 1) :

Table 1 : Contaminants watched in 2003

	Seeds	Meal	Oil
Pesticides	Organophosphorous Organochlorous Pyrethrinoids	Organophosphorous Organochlorous Pyrethrinoids	Organophosphorous Organochlorous Pyrethrinoids
Heavy metals	Cadmium, lead	Cadmium, lead	-
Mycotoxins	Aflatoxin B1	Aflatoxin B1	-
Salmonellas	-	*Salmonellas*	-
PAHs	-	-	PAHs

Concerning the seeds, pesticides were detected in 8% of total samples. Nevertheless, the analysis didn't detect the same molecules in oil and seeds. It is likely that this difficulty is due to the lack of accurate method for seeds.

Lead was not detected in seeds and cadmium concentration was widely below the MRLs.

Afatoxin B1 was not detected.

Concerning the meal, *salmonellas* was detected in 4 samples. Pesticides residues and lead were not detected. Cadmium was detected below the MRLs.

Conclusion

The survey plan of the French oilseed food chain is still in a training phase. Nevertheless, the communication about this plan has to be developed for increasing the memberships all along the oilseed network and to improve the representativity of the collected data. Moreover, it will be necessary, in a second stage, to target the plan at specific contaminants and to have a good knowledge of the sampling conditions.

Acknowledgments

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References

Darracq, S., **2004**. – La sécurité sanitaire : une priorité pour la filière oléagineuse. Oléoscope, **74**, février, p. 34-36.

Delplancke, D., **2000**. – Sécurité sanitaire des aliments : de la nécessité d'une approche de filière. OCL, vol. 7

European Community Commission, 2000, White Paper on food safety.

Lacoste, F., Dauguet, S., Soulet, B., Arnaud, J.N., Brenne, E., Food safety in the field of vegetable oils : from monitoring if undesirable compounds to survey plans. Modern aspects of fats and oils, 26th world congress and exhibition of the international society for fat research (ISF), September **2005**.

High Oleic, low linolenic (HOLL) specialty canola development in Australia

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Abstract

The development of High Oleic, Low Linolenic acid (HOLL) canola is a high priority for the Australian oilseed industry. HOLL canola oil, with >65% oleic acid content and <3% linolenic acid content, has increased oxidative stability compared with conventional canola oil. The demand for a stable, healthy oil that can replace hard fats commonly used in the fast food industry has increased with the mandatory labelling of trans fat content and an increased health awareness being observed in many parts of the world. Of the more than 40 cultivars currently available in Australia, only 5 are HOLL specialty canola, and most in limited availability. This number is expected to increase in coming years, with the development of herbicide tolerant and hybrid HOLL cultivars. It has been estimated that in the domestic market more than 50,000 tonnes of oil could replace hard fats that are currently imported. This would be worth an extra $25 million annually to the oilseed industry in Australia. The Department of Primary Industries – Victoria, in collaboration with Cargill Specialty Canola Oils, are working on developing agronomically suitable HOLL cultivars for Australia. The first two cultivars from this collaborative program were released in 2006, with two replacement cultivars now in commercial seed production. Cargill 102 and Cargill 103 (due for release in 2007) have yields competitive with other conventional mid canola cultivars combined with high provisional blackleg ratings.

Key words: Specialty oils, HOLL, *trans* fatty acids, hydrogenation, deep frying, oxidative stability, yield, blackleg

Introduction

Australian oilseed breeders are seeking to ensure the sustainability and competitiveness of the Australian canola industry through the development of specialty oilseed *Brassica* cultivars with a range of maturities adapted to diverse growing regions of Australia. The Australian domestic market is beginning to see a trend towards healthier foods, as major food companies switch from hard oils high in saturated fats, to soft oils low in saturated fats, and high in mono- or poly- unsaturated fats (AOF Strategic Plan, 2005). The production of High Oleic, Low Linolenic acid (HOLL) canola has been identified as a high priority for the industry, as HOLL canola oil offers increased health benefits to alternative oils. In particular the development of HOLL canola suitable to the Australian environment will benefit the industry by enabling replacement of the hard fats previously imported and increase the opportunities for domestic processors. It is estimated that if the current usage of hard fats (palm and tallow) was replaced there could be a market for more than 50,000 tonnes of HOLL canola, worth an estimated $25 million to the Australian Oilseed industry annually (AOF Strategic Plan, 2005). While the availability of such cultivars is currently limited, the increased demand for this product has resulted in an increased focus on developing HOLL canola in Australia.

HOLL Canola

Traditional canola oil is very low in saturated fats (7%). It typically has approximately 60% of the monounsaturated fatty acid oleic acid, with around 20% and 10% of the two polyunsaturated fatty acids linoleic and linolenic acids, respectively. Canola oil is highly popular in the domestic market, and recognised by consumers as a healthy oil for use in their general food preparation. The high level of polyunsaturated fatty acids renders the oil fully liquid at room temperatures. However, as with many highly unsaturated oils, it has decreased oxidative stability compared to oils high in saturated fatty acids (Strayer, 2006). The lack of stability makes the oil unsuitable for use in long term food preservation or deep frying as the fat can become rancid with time, producing distasteful odours and flavours, thus canola oil is not widely used in commercial applications such as the take away food and restaurant industries. Oxidative stability can be increased through partial hydrogenation of the oil whereby solid fats are produced artificially by heating the oil in the presence of metal catalysts (often nickel) and hydrogen (Ascherio *et al.,* 1999, Strayer, 2006). Partially hydrogenated oils have been used as a replacement for animal fats since the 1960s, when research indicated that a high level of saturated fats in the human diet lead to increases in coronary heart disease (Strayer, 2006). In recent years however, it has been recognised that *trans* fatty acids, produced during partial hydrogenation, can have significant impact on our health and may also contribute to the development of coronary heart disease (Willet *et al.,* 1993). Research indicates that *trans* fatty acids, may in fact be as bad, if not worse for human health than saturated fats. There are suggestions that because of the increase in Low Density Lipoprotein (LDL – 'bad' cholesterol), combined with the reduction

of High Density Lipoprotein (HDL – 'good' cholesterol), *trans* fatty acids could be twice as bad for us as saturated fats (Ascherio *et al.,* 1999).

Conventional deep frying oils (e.g. palmolein) are high in saturated fats. Palm oil typically contains 45% palmitic acid, a fatty acid with a high melting point (62.9 ^{o}C) and high oxidative stability. Some market movement toward partially hydrogenated oils (particularly in the snack food and baking industries) has seen a decrease in saturated fats in the diet. The introduction of these oils into food products has exposed the consumer to much higher levels of *trans* fatty acids than they have previously been exposed to when eating meat and dairy products, which contain naturally occurring *trans* fats in very small amounts. With the increased understanding of the role that oils can have on health, consumer trends now show a movement away from saturated fats and a demand for healthy '*trans* fat free' alternatives. The production of oil with increased oxidative stability, low saturated fats, and no *trans* fatty acids would both increase the healthiness of commercially prepared foods and also enable a decrease in imports of foreign oils for use in the Australian food industry.

While conventional canola oil is low in saturated fats, and contains no *trans* fatty acids, its low oxidative stability makes it unsuitable as a replacement oil in the commercial food industry. HOLL canola oil, however, has no trans fatty acids, but has increased oxidative stability, providing an increased frying life (Table 1), without the need for partial hydrogenation.

Table 1. Frying life of different oil products, measured as Active Oxygen Method (AOM) hours

Product	Frying life
	(AOM hours)
Conventional canola oil	12
Conventional soybean oil	12
Hydrogenated soybean	25
CV65 HOLL	30
CV75 HOLL	34
CV85 HOLL	60

HOLL canola oil refers to High Oleic (>65%), Low Linolenic (<3%) acid. It is the combination of these two parameters that increases the oils oxidative stability compared with conventional canola oil (Figure 1). The frying life of a HOLL product is dependent on the percentage of polyunsaturated fatty acid species in the oil. CV 65 HOLL (>65% oleic acid), CV75 (>75% oleic acid) and CV85 (>85% oleic acid) have increasingly longer frying life than conventional canola (Table 1).

Low linolenic canola has been available in Canada since 1988 when the University of Manitoba released 'Stellar'. Oil evaluation revealed that stability was increased by 17.5% in the accelerated oxidation method test (Scarth *et al*., 1988). Stability has been further increased with the combination of high oleic and low linolenic acids – HOLL canola. There are many minor constituents of oils that could effect stability and flavour of the final product, and therefore accelerated aging and sensory testing should also be performed to ensure flavour and stability are as required.

Historically, the narrow gene pool available in the initial cultivars led to reduced yields. Today this problem has been overcome with improved genetic diversity and hybrid development, putting the specialty canola varieties on par with their conventional counterparts.

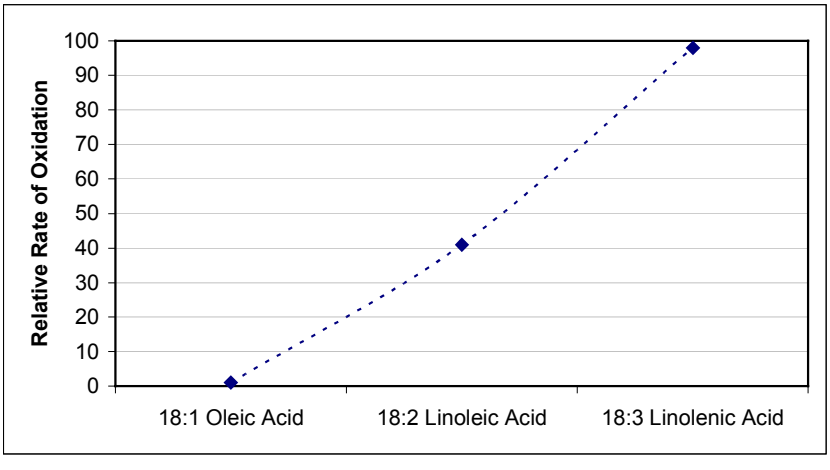

Fig 1. Oxidation rate of different 18 carbon chain fatty acids in conventional canola (values from Frankel, 2005)

Cargill Specialty Canola Oils and Dow AgroSciences now market HOLL canola in Canada, with 1.7 million acres of specialty canola sown in 2005 (Hausmann, 2005) producing about 10% of the Canadian canola production. This is expected to rise to 3 million acres (20% production) in the next 2 – 3 years (Canola Council of Canada, 2006), and that these figures will continue to increase as the awareness of the role of *trans* fat in the diet increases. Currently *trans* fat content must be labelled

separately from other fats on product packaging in the USA and Canada, and the United States Food and Drug Administration (USFDA) recommends a zero daily intake of *trans* fat. Many food manufacturers are in the process of reformulating existing brands with HOLL canola oils, and it is expected than many new products will become available over the next several years. In Denmark no foodstuffs may be sold which contain more than 2 grams of *trans* fat per 100g of fat (i.e. no more than 2% *trans* fat) (Stender and Dyerberg, 2003), and the findings from the Danish Nutrition Council have been submitted to the EU for consideration. To date New Zealand and Australia have no *trans* fat labelling requirement and only require that the source of fats (animal or vegetable) be listed (FSANZ, 2005).

In Australia three HOLL canola cultivars were commercially grown in 2006. NMC 130 was bred by Nutrihealth, a subsidiary of Nufarm, and released in 2006, with an expected production of 20,000 tonnes this year (Gororo, pers. comm.). The product, marketed as MONOLA is available at supermarkets, and is used in some commercial food preparation ventures. NMC 130 contains 67-72% oleic acid with less than 3% linolenic acid and in 2005 National Variety Trials (NVT) averaged 110% of ^{AV}SAPPHIRE, an Australian mid maturing conventional canola. Nutrihealth expects to release two new HOLL cultivars in 2007, including one with triazine tolerance.

CARGILL 100 and CARGILL 101 were developed as part of a collaborative breeding program in Australia between Cargill Specialty Canola Oils (CSCO) and the Department of Primary Industries Victoria (DPI-Vic). These cultivars were available under limited release in 2006 and were grown in southern NSW under an Identity Preservation (IP) contract. Growers of these lines were paid a premium for delivery of HOLL quality oil, and to allow for the slightly reduced yields compared with conventional mid maturity cultivars. Two replacement cultivars are due for release in 2007. In multi-location trials in Victoria during 2005, the two new cultivars – CARGILL 102 and CARGILL 103 - averaged 100% and 108% of ^{AV}SAPPHIRE. They both have good blackleg resistance with provisional ratings of 8.5 and 8.0 respectively on a 0-9 scale with "0" susceptible and "9" most resistant. Yield, blackleg resistance and oil quality are key breeding priorities for the collaborative program, which expects to have hybrid cultivars for release in 2008, and herbicide tolerant cultivars in the following years.

The Department of Agriculture and Food, Western Australia (AgWA) are also working on the development of HOLL canola suited to Australian conditions. AgWA has one cultivar ready for commercial release, and is in the early stages of developing triazine tolerant HOLL canola (Walton, pers. comm.).

Following extensive multi-site, multi-year agronomic studies, mid season environments with warm ripening temperatures and adequate moisture during seed filling were identified as the major target area for HOLL production. These conditions are required to ensure minimum fatty acid specifications are met on a regular basis. The development of herbicide tolerant and hybrid HOLL cultivars will also increase the potential area of adaptation for HOLL canola. Contract production of HOLL cultivars under identity preservation is also crucial to maintain product quality.

Given the direction the major global food organisations are going, and the current interest in specialty oil canola in Australia, it is expected that the area of HOLL production will continue to increase. The increase is likely to be steady, rather than spectacular, with perhaps 10% of the Australian crop being HOLL cultivars within 5-7 years (White, pers. comm.). This will create a significant market opportunity for Australian growers. The ultimate relative importance of HOLL compared with conventional canola will depend on market demand.

References

Ascherio, A., Stampfer, M. J., and Willett, W. C. (1999). Trans fatty acids and coronary heart disease. Background and scientific review. http://www.hsph.havard.edu/reviews/transfats.html

Australian Oilseeds Federation (AOF) Strategic Plan 2010, Australian Oilseed Industry Review 2015. (2005). Strategic Plan Booklet, Australian Oilseeds Federation Forum, Sydney, Australia, 12 October 2005.

Canola Council of Canada. (2006). Canola facts: next generation of healthy products. http://www.canola-council.org/facts_nextgen.html

Frankel, E.N. (2005). Lipid Oxidation. The Oily Press, Bridgwater, England.

Food Standards Australia New Zealand (FSANZ). (2005). The Australia New Zealand Food Standards Code. http://www.foodstandards.gov.au/foodstandardscode

Hausmann, C. (2005). Canola: competing in the world food market. Canola Council of Canada. http://www.canola-council.org/ccc_proceed_2005.html

Scarth, R., McVetty, P. B. E., Rimmer, S. R., and Stefansson, B. R. (1988). Stellar low linolenic – high linoleic summer rape. Can J. Plant Sci. **68**, 509-511.

Strayer, D. (Ed), (2006). Food Fats and Oils. Institute of Shortening and Edible Oils, Washington.

Stender, S., and Dyerberg, J. (2003). The influence of *trans* fatty acids on health. 4th Edition. Publ. No. 34 – the Danish Nutrition Council.

Willet, W.C., Stampfer, M.J., Manson, J. E., Colditz, G. M., Speizer, F. E., Rosner, B. A., and Hennekens, C. H. (1993). Intake of *trans* fatty acid and risk of coronary heart disease among women. Lancet **341**, 581-585.

Nutritional value of cruciferous oilseed crops in relation to profile of accumulated biomolecules with especial regard to glucosinolate transformation products

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Abstract

Cruciferous oilseed crops accumulate relatively high concentrations of nutritional high quality oil and proteins in their seeds. In addition to these major seed components, their co-occurrence with high concentrations of dietary fibre (DF) and various bioactive components as glucosinolates/glucosinolate products is decisive for the nutritional value of the seed meal or products obtained from it. Depending on structural types and concentration of glucosinolates and glucosinolate derived products, these compounds can be either health beneficial or act as antinutrients. The effects of these components depend, however, strongly on the type of animal and development of the animals fed with the diets based on these compounds. Results from studies based on differently treated and processed seeds and from use of individual isolated seed components included in standard diets are evaluated and treated in relation to literature data as a basis for recommendations of acceptable concentrations of glucosinolates/glucosinolate products in animal diets. The relation between these recommendations of acceptable concentrations in feed to different animals and those reported as necessary for plant pathogen control (biofumigation) and health beneficial effects (chemoprotection) is discussed.

Introduction

The nutritional value of cruciferous oilseed crops has been a hot subject for a long time, with research being intensified some decades ago as a result of the success plant breeders had in connection with the production of double low oilseed rape (Bunting, 1981; Sørensen, 1985). Advantages of double low oilseed rape cultivars are related to the opportunities for an optimal utilization of relatively high yielding oil- and protein rich agricultural crops with ca. 45 % oil and 20 % protein in seed dry matter (DM), and less than 0.5% erucic acid in the oil and less than 20 μmole of glucosinolates per g. of seed DM (GCIRC, conf., 2003). Both of these major seed components have a high nutritional value and quality if problems caused by dietary fibres (DF) and especially glucosinolates can be solved (Bille et al. 1983a; 1983b; Bjergegaard et al. 1991). This gives the need for consideration of effects caused by processing and biorefining (Bagger et al. 2007) as well as the need for taking into account the variation in effects caused by structurally different glucosinolates and glucosinolate derived products (Bille et al. 1983; Bjerg et al. 1987a; Loft et al. 1992; Bonnesen et al. 1999; Bjergegaard et al. 2000; Vang et al. 2001). The great variation in structurally different glucosinolate derived products reflects primarily the glucosinolate structures (Bellostas et al. 2007) and the reaction conditions during non-enzymatic and enzyme/myrosinase (EC 3.2.1.147) catalysed reactions (Bjergegaard et al. 1994; Agerbirk et al. 1998; Bjergegaard et al. 1999; Buskov et al. 2000a; 2000b; 2000c; Barba et al. 2005; Bellostas et al. 2006). Thereby, it is also expected and found that effects on:

- feed and food quality,
- animal or human health and diseases and
- the value of final products,

are a function of structure and concentrations for both the glucosinolates and products thereof (Bille et al., 1983b; Bjerg et al., 1989; Jensen et al., 1991; Michaelsen et al., 1994; Bonnesen et al., 1999; Bjergegaard et al., 2001; Vang et al., 2001; Bellostas et al., 2007).

Materials and Methods

Details and comprehensive descriptions of the applied analytical methods are presented elsewhere (Sørensen et al., 1999; Sørensen 2001; Bellostas et al., 2003; Bellostas et al., 2006) as are applied procedures for evaluations of nutritional values and effects of allelochemicals/xenobiotics, N-balance trials with rats (Bille et al., 1983b; Bjerg et al., 1989), mink trials (Danielsen et al., 1987), young bull trials (Andersen and Sørensen, 1985) and evaluations of health and anticarcinogenic effects (Loft et al. 1992; Bonnesen et al., 1999; Vang et al., 2001).

Results and Discussion

Cruciferous oilseed crops are important sources for both vegetable oil (40-45% of seed DM) and protein (20-25 % of seed DM). Oil from these crops has been used as energy sources from ancient times, and in the recent years, additional focus has been directed at this oil for its use as fuels of biodiesel (Knothe, 2006; GCIRC conf., 2003). With introduction of double

low oilseed rape, an increased interest in the oil has been focused on the nutritional applications, as it has been found to be high quality oil (Bunting, 1981; Sørensen, 1985; GCIRC conf. 2003). The result of a large and increasing production of oils from cruciferous seeds is thus a great amount of deoiled meal for applications as feed, food and non-food, where the main limitations in its uses are caused by dietary fibres (DF, Bjergegaard et al. 1991) and especially glucosinolates and glucosinolate derived products. The lipophilic and volatile glucosinolate derived products may also create environmental (smell, taste, toxicity) and oil-quality problems, depending on structures of the glucosinolate products (Sørensen, 2001), which again is linked to the plant source and glucosinolate type. Cruciferous plant sources most often used belong to genera of the family Brassicaceae, and these sources are quantitatively dominated by Brassica cultivars; *B. napus* and *B. rapa* (Table 1).

Table 1. Quantitatively dominating seed glucosinolates in different Brassicaceae species.

Species	Glucosinolate dominating
Brassica napus L. *B. rapa* L. *B. oleracea* L.	10-16 different gluosinolates (Bjerg et al., 1987b)
B. nigra (L.) Koch *B. carinata* Braun *B. juncea* (L) Czern & Coss	Sinigrin (Bellostas et al., 2006)
Crambe abyssinica Hoechst. ex R.E. Fries	(2S)-2-Hydroxybut-3-enylglucosinolate
Barbarea vulgaris R. Br.*	(2S) and (2R)-2-hydroxy-2-phenylethylglucosinolate
B. verna (Miller) Ascherson*	Phenethylglucosinolate
Lepidium campestre (L.) R. Br.*	Sinalbin
Camelina sativa (L.) Crantz	(R)-Methylsulphinyl-$(CH_2)_n$-glucosinolates#
Sinapis alba L.	Sinalbin

Ref. (* Andersen et al., 1999; Sørensen, 2001) # Dominating glucosinolates with n=9 and 10.

The cruciferous meal available for feed, food and non-food applications after deoiling contains thus various types and concentrations of glucosinolates defined by the applied type of seeds, and depending on storage and processing conditions a great number of glucosinolate derived products can be present in the meal. When applied at few percent in diets to monogastric animals, this will give reduced nutritional value for the cultivars with more than 20 μmol/g seed DM. A great number of oilseed rape cultivars – even double low oilseed rape – also have a level and composition of glucosinolates that give nutritional problems (Bille et al., 1983; Bjerg et al., 1987; Jensen et al., 1991).

The acceptable level of glucosinolates in diets to monogastric animals needs to be below 2nd level (Table 2) or 1-2 μmol/g DM to avoid nutritional problems. However, as described elsewhere (Bjerg et al., 1987), the acceptable level depend on the type of animal, its age/development and especially the structure of the glucosinolate/glucosinolate products.

Table 2. Glucosinolate level in seed, seed meal and diets (DM) to monogastric animal without the presence of glucosinolate transformation products

	level 1**	level 2	level 3''	level 4
Diets (μmol/g DM)	0.5	2.5	12.5	as level 2 but with active myrosinase added
Feed with 20 % rapeseed meal: (μmol/g rapeseed meal)	2.5	12.5	62.5	
Seed before deoling: (μmol/g seed; depend on oil and water content)	1-2	6-8	30-40	

** Generally safe. '' Create nutritional problems.

The quantitatively dominating glucosinolates in double low oilseed rape are (2R)-2-hydroxybut-3-enylglucosinolate (progoitrin) and 4-hydroxyglucobrassicin, which as result of hydrolysis give (5S)-5-vinyloxazolidine-2-thione (goitrin) and thiocyanate ion, respectively. Both hydrolysis products have strong effect on the thyroid gland and thereby on the metabolism, growth and development of the animals. The indolyl group of 4-hydroxyglucobrassicin gives, as result of transformations, appreciable negative effects on rapeseed products caused by a complex group of structurally different compounds (Jensen et al. 1991; Bellostas et al., 2007). Appreciable variations are also found for other types of glucosinolate transformation products as isothiocyanates (ITC's; R-N=C=S) and nitriles (RCN):

Table 3. LD_{50} of glucosinolate hydrolysis products on rats and mice.

Glucosinolate precursor	Compound	Animal	LD_{50} mmol/kg	References
Sinigrin	allyl-ITC	rats	1	Langer& Geer 1968
Glucoiberin	CH_3-SO-$(CH_2)_3$-ITC	rats	0.5	Langer& Geer 1968
Phenethyl-glucosinolate	Phenethyl-ITC	rats	0.3-0.5	Langer& Geer 1968
Epiprogotrin	(5R)-5-vinyloxazaolidine-2-thione	mice	11-13	Van Etten et al., 1969
Epiprogotrin	1-cyano-2-hydroxy-3,4-epithiobutane	rats	1.5-2.0	Van Etten et al., 1969
Epiprogotrin	1-cyano-3,4-epithiobutane	rats	0.8	Van Etten et al., 1969
Epiprogotrin	(2S)-1-cyano-2-hydroxybut-3-ene	rats	2.2-2.4	Van Etten et al., 1969
Glucobrassicin	Indol-3-ylacetonitrile	rats	1.1	Fenwick et al., 1983

Comparing the concentration levels and structures of glucosinolates and glucosinolate derived products acceptable for nutritional purposes and those needed for food, feed and plant protection (Bellostas et al., this conference; 2007) gives the basis for progress in these fields.

Conclusion

Glucosinolates and transformation products of glucosinolates are key factors for the nutritional value of cruciferous oilseed crops and for the value of products produced from them. Considering the physiological effects of glucosinolates and glucosinolate products it seems to be possible to use appropriate structural types in concentrations of the compounds where they can function as food, feed and plant protection agents and/or as chemoprotection or health beneficial compounds without reaching the toxic levels of the compounds.

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References

Andersen, A.A.M., Merker, A., Nilsson, P., Sørensen, H., and Åman, P.: Chemical composition of the potential new oilseed crops Barberea vulgaris, Barbarea verna and Lepidium Campestre. J. Sci. Food Agric. (1999) 79, 179-186

Andersen, H.R. and Sørensen, H.: Double low rapeseed meal in diets to young bulls. In: Advances in the Production and utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster (1985) 11, 208-217.

Agerbirk, N., Olsen, C.E. and Sørensen, H.: Initial and final products, nitriles and ascorbigens produced in myrosinase catalysed hydrolyses of indole glucosinolates. J. Agric. and Food Chemistry (1998), 46 (4), 1563-1571.

Barba, I., Bellostas, N., Puiggros, A., Sørensen, J.C., Sørensen, S. and Sørensen, H.: Myrosinases, glucosinolates and transformation products in Brassica, Raphanus and Sinapis: Physico-chemical properties and activity of myrosinase isoenzymes. GCIRC Bulletin (2005) 22, 6pp.

Bellostas, N., Jørgensen, A.L.W., Lundin, N.V.F., Petersen, I.L., Sørensen, H., Sørensen, J.C., Sørensen, R. and Tidmand, K.D.: Comparison of physico-chemical properties of myrosinase isoenzymes occuring in seeds of Brassica species of the U triangle. In: Proceedings of 11th International Rapeseed Congress, 6-10 July, Copenhagen, Denmark. ISBN 87-7611-003-6 (2003) PP25, 720-723.

Bellostas, N., Sørensen, A.D., Sørensen, J.C. and Sørensen, H.: Genetic variation and metabolism of glucosinolates in cruciferous oilseed crops. In: Rapeseed Breeding: Advances in Botanical Research (Ed. Dr. Surinder Gupta) Academic Press/ Elsevier, Dan Diego, Vol. 54; 2007.

Bellostas, N., Sørensen, J.C. and Sørensen, H.: Micellar electrokinetic capillary chromatography – Synchronous monitoring of substrate and products in the myrosinase catalysed hydrolysis of glucosinolates. J. Chromatogr. A. (2006), 1130, 246-252.

Bellostas, N., Sørensen, J.C. and Sørensen, H.: Profiling glucosinolates in vegetative and reproductive tissues of four Brassica species of the U-Triangle for their biofumigation potential. J. Sci. Food Agric. (2007) JSFA-05-0807R1 in press.

Bille, N., Eggum, B.O., Jacobsen, I., Olsen, O. and Sørensen, H.: The effects of processing on antinutritional rape constituents and the nutritive value of double low rapeseed meal. Zeitschr. Tierphysiol., Tierernährung u. Futtermittelkd. (1983a) 49, 148-163.

Bille, N., Eggum, B.O., Jacobsen, I., Olsen, O. and Sørensen, H.: Antinutritional and toxic effects in rats of individual glucosinolates (±myrosinases) added to a standard diet. 1. The effects on protein utilization and organ weights. Zeitschr. Tierphysiol., Tierernährung u. Futtermittelkd. (1983b) 49, 195-210.

Bjerg, B., Eggum, B.O., Rasmussen, K.W. and Sørensen, H.: Acceptable concentrations of glucosinolates in double low oilseed rape and possibilities of further quality improvements by processing and plant breeding. In: 7th International Rapeseed Congress, Poznan Poland, (1987a), VII, Vol. 7, 1619-1626.

Bjerg, B., Eggum, B.O., Jacobsen, I., Otte, J. and Sørensen, H.: Antinutritional and toxic effects in rats of individual glucosinolates.(myrosinases) added to a standard diet (2). Zeitschr. Tierphysiol., Tierernährung u. Futtermittel-kd. (1989) 61, 227-244.

Bjerg, B., Larsen, L.M. and Sørensen, H.: Reliability of analytical methods for quantitative determination of individual glucosinolates and total glucosinolate content in double low oilseed rape. In: 7th International Rapeseed Congress, Poznan Poland, (1987b), IV, Vol. 6, 1330-1341.

Bjergegaard, C., Buskov, S., Ib, K., Sørensen, H., Sørensen, J.C. and Sørensen, S.: Effects of various antioxidants and storage conditions on oxidative degradation of the indole glucosinolate 4-hydroxyglucobrassicin. In: Biologically-active Phytochemicals in Food. Analysis, Metabolism, Bioavailability and Function. The Royal Society of Chemistry, UK. ISBN 0-85404-806-5 (2001), 88-90.

Bjergegaard, C., Buskov, S., Sørensen, H., Sørensen, J.C., Sørensen, M. and Sørensen, S.: Reactions between glucosinolate products and thiol groups in food components. Czech. J. Food Sci. (2000) 18, 28-30.

Bjergegaard, C., Eggum, B.O., Jensen, S.K. and Sørensen, H.: Dietary fibres in oilseed rape: Physiological and antinutritional effects in rats of isolated IDF and SDF added to a standard diet (1). J. Anim. Physiol. Anim. Nutr.(1991) 66, 69-79.

Bjergegaard, C., Li, P.W., Michaelsen, S., Møller, P., Otte, J. and Sørensen, H.:Glucosinolates and their transformation products - compounds with a broad biological activity. Bioactive Substances in Food of Plant Origin (1994) 1, 1-15.

Bjergegaard, C., Møller, P., Sørensen, H., Sørensen, J.C. and Sørensen, S.: Micellar electrokinetic capillary chromatography of thiocarbamoyl derivatives produced in reactions between isothiocyanates and amino acids. J. Chrom. A (1999) 836, 115-127.

Bonnesen, C., Stephensen, P.U:, Andersen, O., Sørensen, H. and Vang, O.: Modulation of cytochrome P-450 and glutathione S-transferase isoform expression in vivo by intact and degraded indolyl glucosinolates. Nutr. Cancer (1999) 33(2), 178-187.

Bunting, E.S. Production and Utilization of Protein in Oilseed Crops Martinus Nijhoff Publ., The Hague/Boston/London (1981) 5.

Buskov, S., Olsen, C.E., Sørensen, H. and Sørensen, S.: Supercritical fluid chromatography as basis for identification and quantitative determination of indol-3-ylmethyloligomers and ascorbigens. J. Biochem Biophys Methods (2000a) 43, 307-327.

Buskov, S., Hasselstrøm, J., Olsen, C.E., Sørensen, H., Sørensen, J.C., and Sørensen, S.: Supercritical fluid chromatography as method of analysis for determination of 4-hydroxybenzylglucosinolate degradation products. J. Biochem. Biophys. Methods (2000b) 43, 281-298.

Buskov, S., Hansen, L.B., Olsen, C.E., Sørensen, J.C., Sørensen, H. And Sørensen, S.: Determination of ascorbigens in autolysates of various Brassica species using supercritical fluid chromatography. J. Agric. Food. Chem. (2000c) 48(7), 2693-2701.

Danielsen, V., Eggum, B.O., Rasmussen, K.W. and Sørensen, H.: Long-term studies of requirements to the quality of rapeseed meal from double low varieties used in sow diets. In: 7th International Rapeseed Congress, Poznan Poland, (1987), V, Vol. 7, 1727-1734.

Eggum, B.O., Just, A. and Sørensen, H.: Double low rapeseed meal in diets to growing - finishing pigs. In: Advances in the Production and utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster (1985) 11, 167-176.

GCIRC Conf., 2003: Proceedings of 11th International Rapeseed Congress, Copenhagen, ISBN 87-7611-003-6

Henriksen, P., Hillemann, G., Mortensen, K. and Sørensen, H.: Requirements to the quality of oilseed rape which can be used without problems in diets to mink (Mustela vison Screb.). In: 7th International Rapeseed Congress, Poznan Poland, (1987), VII, Vol. 7, 1817-1824.

Jensen, S.K., Michaelsen, S., Kachlicki, P. and Sørensen, H.: 4-Hydroxyglucobrassicin and degradation products of glucosinolates in relation to unsolved problems with the quality of double low oilseed rape. GCIRC - Congress, Saskatoon, Canada. (1991), V, 1359-1364.

Knothe, G. Biodiesel and vegetable oil fuels: Then and now. Inform, 2006, 17 (11), 729-731.

Loft, S., Otte, J., Poulsen, H.E. and Sørensen, H.: Influence of intact and myrosinase-treated indolyl glucosinolates on the metabolism in vivo of metronidazole and antipyrine in the rat. Fd. Chem. Toxic, (1992), 30 (11), 927-935.

Michaelsen, S., Otte, J., Simonsen, L.-O. and Sørensen, H.: Absorption and Degradation of Individual Intact Glucosinolates in the Digestive Tract of Rodents.

Acta Agric. Scand. (1994) Sect. A, 44, 25-37.

Petersen, I.L., Andersen, K.E., Sørensen, J.C. and Sørensen, H.: Determination of shikimate in crude plant extracts by micellar electrokinetic capillary chromatography. J. Chromatogr. A. (2006), 1130, 253-258.

Sørensen, H.: Advances in the Production and utilization of Cruciferous Crops. (Ed. Sørensen, H.) Martinus Nijhoff/Dr. W. Junk Publ., Dordrecht/Boston/Lancaster (1985) 11.

Sørensen, H., Sørensen, S., Bjergegaard, C., Michaelsen, S.: Chromatography and capillary electrophoresis in food analysis.

Royal Society of Chemistry, UK, (1999) 470 pp, ISBN 85404-561-9

Sørensen, J.C. Biorefined oilseed products – high quality products with added value. Ph.D. Thesis 2001, Chemistry Department, KVL, Frederiksberg C, Denmark. 293pp.

Vang, O., Frandsen, H., Hansen, K.T., Sørensen, J.N., Sørensen, H. and Andersen, O.: Biochemical effects of dietary intakes of different broccoli samples. I. Different modulation of cytochrome P-450 activities in rat liver, kidney and colon. Metabolism. Clinical and Experimental (2001) 50(10), 1123-1129

Distribution of n-7 Fatty acid in *Brassicacea.*

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Abstract

Cis Vaccenic acid, a C18:1 (*n-7*) isomer of oleic acid (C18:1 (*n-9*)) has been found in several oilseeds. It is synthetized from palmitic acid (C16:0) via production C16:1 (*n-7*) by a Δ9 desaturase and elongation by an elongase giving C18:1 (*n-7*). While this fatty acid does not have a known antinutritional effect, there has been some interest in it, particularly in relation to its *trans* form *t*-vaccenic acid.

Brassica contained both (*n-9)* and (*n-7)* fatty acids for the C18:1, isomers of C20:1 and C22:1 fatty acids were also found. The amount of the C18:1(*n-7*) isomer varied from the seed sources.

Brassica specie relations have been established using electrophoretic studies of the proteins protein and chromosome pairing. This project assessed the use of *n-7* fatty acid isomers as common characteristic of several Brassica species and tried to establish if these fatty acids could be used as genetic markers.

Introduction

The cabbage or mustard family (*Brassicaceae*) includes over 3000 species, grouped in over 300 genres. They included weeds or domesticated plants grown as vegetables, ornamental flowers or for seeds. Middle East is the presumptive point of origin of the *Brassica* species but now they appear as cultivated plants or weeds in Europe, North and South America, and Australia. Seeds of the various *Brassica* species had very different relative fatty acid compositions; differences that have been amplified in the recent years by breeding to produce specialty oils. In our studies of the fatty acic composition of the oil from the weed, wild mustard or charlock (*Sinapis arvensis)*, (*n-9*) and (*n-7*) isomers for C18:1, C20:1and C22:1 fatty acids were identified. These fatty acids had been previously been identified in *B. napus* and *B. rapa (campestris)* (Applequist, 1969) and the (*n-7*) isomer of oleic acid was associated with structural lipids in *B. rapa* (Cv. Tobin) and *B. napus* (Cv. Westar) (Hu *et al*., 1994). However, there is little information on the distribution of (*n-7*) isomers of longer chain fatty acids in different *Brassica* species.

Materials and methods

Materials:

Samples: *Brassica* samples obtained from Mr. R.K. Gugel, curator of the Crucifer Node of the Plant Gene Resources of Canada included *Brassica carinata* (SRS1578, Dodolla, S67, PAK85490, SRS1460), *Brassica juncea* (Donskaja, Lethbridge 22A, Cutlass, Varuna, AC Vulcan, J197-102), *Brassica napus* (Argentine, AC Excel, Golden, Westar and Midas), *Brassica nigra* (SRS190, SRS586, SRS1170, SRS195), Brassica rapa (AC Parkland, Echo, Polish, R500, Torch), *Brassica tournefortii* (SRS349, PAK85655, SRS3036, SRS3038, SRS3043), *Camelina sativa* (SRS933), *Crambe abyssinica* (Prophet), *Eruca sativa* (PAK856392, PAK85886, PAK85889, PAK85873, PAK85896), *Raphanus sativus* (Nemex, Rauola, Zenit, IDC3098, SRS1078), *Sinapis alba* (Tilney, Ochre, Gisilba, Andante, AC Pennant) and *Sinapis arvensis* (SRS3100).

Reagents and Standards: Methanolic base was from Sigma (Sigma-Aldrich Canada Ltd., Ont. Canada). A gas chromatography reference standard, designed for this project was obtained from Nu Chek Prep Inc. (Elysian, MN, USA).

Fatty acid methyl esters: Samples (10 seeds) were used to prepared FAMEs using sodium methoxide as catalyser.

GC analysis: FAMES were analyzed using a Hewlett Packard 5890 gas chromatograph (Agilent Technologies, Mississauga, Ont., Canada) equipped with a flame ionization detector and a 7673A injector tower and a Agilent 6890N Network GC System with a 5973 inert Mass Selective Detector and equipped with a 7683B Autoinjector Module using the same temperature program. Methyl esters were separated on a Suplecowax 10 silica column (Sigma-Aldrich Canada Ltd, Mississauga, Ont., Canada) (60 m x 0.32 mm, 0.25 μm). Hydrogen was the carrier gas (2.5 mL/min), injection port temperature was 280°C and detector temperatures were kept at 300°C. The temperature program was as follows: the initial 190°C temperature was maintained for 3 min, a first gradient was made from 190 to 210°C at 2°C/min, then to 280°C at 20°C/min, the final temperature 280°C was held for 3 min for a total run time of 24 min.

Statistical analysis: The statistical analyses were performed using Origin® 6.0 (Microcal Software Inc., Northampton, MA, USA), InStat 3.05 (GraphPad Software Inc., San Diego, CA, USA) and SAS 9.1.3 (SAS Institute INC, Cary, NC, USA).

Results and discussion:

Cultivars of several species of Brassica were used in this study. Quintuplicate FAMEs were prepared for each sample; all were analyzed by GC (Figure 1) and one sample of each quintuplicate FAME was analyzed by GC-MS to allow a correct

identification of the fatty acids. The cultivars had very different relative fatty acid compositions (Table 1); both isomers (*n-9*) and *(n-7*) were found for the C18:1 (oleic acid), the C20:1 (eicosenoic acid) and the C22:1 (erucic acid) fatty acids although in different proportions

Cis-vaccenic acid (11-*cis*-octadecenoic acid or C18:1(*n-7*)), an isomer of oleic acid (9-*cis*-octadecenoic acid or C18:1(*n-9*)), represented from 0.4 to 3.4% of the total relative fatty acid contents of the seeds. Some C20:1(*n-7*) and C22:1(*n-7*) isomers were also found. However, their levels were lower than C18:1(*n-7*) levels; they varied from below the limit of detection to 1.6% and below the limit of detection to 1.3% for C20:1 (*n-7*) and C22:1 (*n-7*), respectively. The percent of (*n-7*)/(*n-9*) varied depending on the species and sometimes the varieties. The ratios ranged from 2.3 to 17.9%, 0 to 34.2% and 0 to 3.9% for C18:1, C20:1 and C22:2, respectively.

The (*n-7*)/(*n-9*) ratios for C18:1, C20:1 and C22:1 were compared to establish if the (*n-7*) fatty acid isomers might a common characteristic of several *Brassica* species (Table 2**).** Unfortunately, *S. arvensis*, *C. abyssinica* and *C. sativa* could not be used in these analyses since only one cultivar of each of these species was available for the study. The (*n-7*)/(*n-9*) ratios for C18:1 showed less variation than the (*n-7*)/(*n-9*) ratios for C20:1 and C22:1 within a species (Table 2); it was used to see if similarities or differences could be observed between the tested species. The C18:1 (*n-7*)/(*n-9*) ratios of *B. carinata, B. juncea* and *B nigra* were statistically different than the C18:1 (*n-7*)/(*n-9*) ratios for all the other tested species (Table 3). In contrast, *B. napus and B. tournefortii* were the species that had C18:1 *(n-7)/(n-9)* ratios statistically similar to the largest number of the tested Brassica species (Table 3). *B. napus* presented a C18:1 (*n-7*)/(*n-9*) ratio statistically similar to *B. rapa*, *E. sativa* and *B. tournefortii*. The C18:1 *(n-7)/(n-9)* ratio of *B. tournefortii* was similar to the one of *B. rapa*, *B. napus*, *R. sativus and S. alba*.

The evolution the *Brassicacae* followed the triangle of U theory, with *B. napus* (n = 19), an amphidiploid species, resulting from crosses between *B. campestris* (*rapa*) (n = 10) and *B. oleracea* (n = 9). *B. juncea*, another amphidiploid species, resulted from crosses between *B. campestris* (*rapa*) (n = 10) and *B. nigra* (n = 8) (U, 1935). Phylogenetic studies of *Brassica* could be contradictory. If the C18:1 *(n-7)/(n-9)* ratios were used to for the chemotaxonomy the tested Brassica, B. *napus, B. rapa, B. tournefortii* and *E. sativa* would be related and *B. tournefortii, R. sativus* and *S. alba* would be also related (Figure 2) It has been shown that *E. sativa* and *B. napus* belonged to the rapa/oleacea lineage along with *R. sativus* (Warwick and Black, 1991). *S. alba* and *B. tournefortii* with ratios similar would belong to the same group; these two species belonged to the nigra lineage (Warwick and Black, 1991). However they had ratios similar to *R. sativus* which was part of the rapa lineage (Warwick and Black, 1991). According to nuclear RFLP studies, *R. sativus* was closely related to *B. nigra* whereas chloroplast and mitochondria DNA restriction site analyses showed that *R. sativus* was closely related to *B. rapa/oleracea* (Yang *et al*., 1998).

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References

Appleqvist, L. (1969). Lipid in Cruciferae: IV. Fatty acid patterns in single seeds and seed populations of various cruciferae and in different tissues of *Brassica napus* L. Hereditas, 61(2), 9-44.

Hu, X., Daun, J.K. and Scarthe, R. (1994). Proportion of C18!n-9 fatty acids in canola seed coat surface and internal lipids. JAOCS, 71(2), 221-222.

U, N. (1935). Genome analysis in *Brassica* with special reference to the experimental formation o Brassica napus and peculiar mode of fertilization. Jpn Bot., 7, 389-452

Warwick, S.I. and Black, L.D. (1991). Molecular systematics of Brassica and allied genera (suntribe Brassicinae, Brassiceae) – chloroplast genome and cytodeme congruence. Theor., Appl. Genet., 82, 81-91.

Yang, Y.W., Tseng, P.F., Tai, P.Y. and Chang, C.J. (1998). Phylogenetic position of Raphanus in relation to Brassica species based on 5S rRNA spacer sequence data. *Bot. Bull. Acad. Sin*., 39, 153-160.

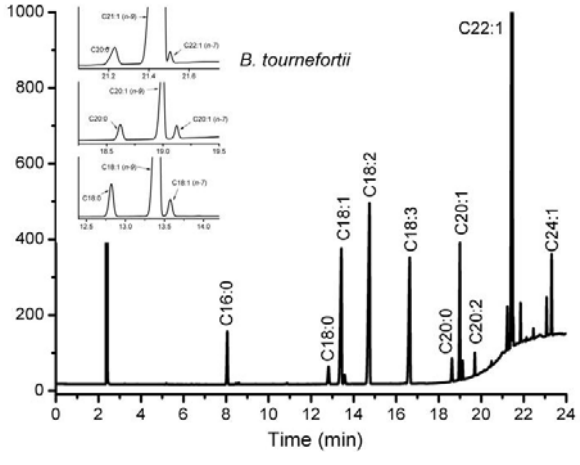

Figure 1: Gas chromatogram of fatty acid methyl esters from *B. tournefortii*. seed showing the presence of both *n*-9 and *n*-7 fatty acids.

Table 1: Relative fatty acid composition (average, n = 5) of the tested Brassica.

Species	Cultivar	Relative fatty acid composition (%)							Ratio (*n-7*/*n-9*) (%)		
		C16:1	C18:1		C20:1		C22:1				
			n-9	*n-7*	*n-9*	*n-7*	*n-9*	*n-7*	C18:1	C20:1	C22:1
B. carinata	Dodolla	0.10	7.50	0.89	5.81	1.01	40.42	0.74	11.93	17.42	1.84
	PAK85490	0.13	7.70	0.90	5.95	1.26	42.36	0.82	11.89	21.31	1.93
	S67	0.11	8.91	0.95	6.27	1.13	41.11	0.76	10.63	18.00	1.85
	SRS1460	0.14	7.36	0.88	6.77	1.18	40.60	0.76	12.08	17.50	1.86
	SRS1578	0.14	7.07	0.96	5.85	1.27	42.39	0.96	13.60	21.72	2.26
B. juncea	AC Vulcan	0.16	18.40	1.64	11.20	0.81	23.35	0.22	8.94	7.26	0.94
	Cutlass	0.15	16.98	1.60	11.20	0.87	24.97	0.24	9.47	7.78	0.94
	Donskaja	0.14	20.35	1.10	9.51	0.63	28.87	0.34	5.41	6.63	1.18
	J197-102	0.21	40.06	2.65	1.73	0.05	0.57	0.00	6.62	2.64	0.00
	Lethbridge 22A	0.19	20.53	1.61	11.30	0.78	22.28	0.26	7.82	6.94	1.18
	Varuna	0.17	9.63	0.76	4.76	1.29	45.86	1.01	7.89	27.25	2.21
B. napus	AC Excel	0.22	60.61	3.28	1.16	0.02	0.03	0.00	5.42	1.95	0.00
	Argentine	0.19	16.42	1.49	11.44	1.30	36.60	0.52	9.11	11.43	1.42
	Golden	0.16	14.84	1.10	9.92	1.40	43.03	0.65	7.46	14.17	1.51
	Midas	0.18	62.98	2.51	1.13	0.02	0.06	0.00	3.99	1.43	0.00
	Westar	0.19	60.80	3.12	1.38	0.01	0.03	0.00	5.13	0.62	0.00
B. nigra	SRS1170	0.27	9.73	1.47	6.61	1.13	34.80	0.91	15.18	17.21	2.61
	SRS190	0.26	9.47	1.46	6.96	1.14	33.63	0.82	15.43	16.45	2.44
	SRS195	0.26	10.23	1.64	7.82	1.23	27.85	0.67	16.14	15.94	1.94
	SRS586	0.24	6.94	1.16	5.39	1.14	38.54	1.01	16.74	21.29	2.62
B. rapa	AC Parkland	0.21	51.09	3.28	0.92	0.02	0.03	0.00	6.42	1.75	0.00
	Echo	0.14	28.80	1.80	10.40	0.61	24.85	0.33	6.24	5.90	1.33
	Polish	0.17	31.98	1.84	10.82	0.57	21.90	0.32	5.78	5.30	1.46
	R500	0.16	11.77	0.55	3.79	1.18	51.95	1.26	4.69	31.42	2.42
	Torch	0.17	58.00	2.56	1.73	0.04	0.72	0.00	4.41	2.11	-
B. tournefortii	PAK85655	0.06	7.94	0.45	5.01	0.66	49.99	0.46	5.63	13.21	0.92
	SRS3036	0.07	8.63	0.45	5.33	0.66	49.70	0.46	5.22	12.32	0.94
	SRS3038	0.07	8.52	0.44	5.48	0.63	49.70	0.53	5.16	11.58	1.07
	SRS3043	0.07	9.55	0.53	5.70	0.71	48.25	0.54	5.58	12.47	1.12
	SRS349	0.08	13.04	0.63	7.84	0.70	44.30	0.51	4.81	8.90	1.16
C. sativa	SRS933	0.07	13.98	0.77	14.13	0.45	3.12	0.03	5.54	3.18	1.04
C. abyssinica	Prophet	0.19	17.52	0.49	3.02	0.89	55.22	1.09	2.83	29.53	1.97
E.. sativa	PAK856392	0.31	12.48	0.94	6.53	1.26	45.52	0.92	7.49	19.40	2.01
	PAK85873	0.25	13.26	0.87	6.83	1.16	46.03	0.77	6.53	17.20	1.68
	PAK85886	0.24	13.89	0.89	8.09	1.01	43.40	0.69	6.38	12.54	1.60
	PAK85889	0.25	13.40	0.84	6.69	1.05	46.65	0.73	6.28	15.75	1.57
	PAK85896	0.25	13.49	0.91	7.98	1.13	44.77	0.70	6.77	14.12	1.57
R. sativus	IDC3098	0.24	32.53	1.55	8.02	0.41	16.09	0.17	4.79	5.07	1.04
	Nemex	0.14	34.93	1.29	7.26	0.19	9.61	0.05	3.71	2.58	0.51
	Rauola	0.17	33.69	1.48	8.84	0.30	12.95	0.07	4.39	3.38	0.52
	SRS1078	0.22	24.68	1.20	9.44	0.64	32.98	0.30	4.87	6.71	0.96
	Zenit	0.17	26.31	0.98	9.61	0.41	27.26	0.16	3.73	4.31	0.57
S. alba	AC Pennant	0.15	26.06	1.04	9.80	0.65	33.30	0.41	3.99	6.65	1.24
	Andante	0.20	24.65	1.30	9.88	0.74	30.49	0.50	5.29	7.47	1.64
	Gisilba	0.17	22.40	1.04	10.01	0.75	36.42	0.53	4.64	7.56	1.46
	Ochre	0.16	25.81	1.05	9.45	0.62	32.76	0.41	4.10	6.60	1.26
	Tilney	0.16	27.93	1.15	8.78	0.62	28.92	0.43	4.13	7.11	1.49
S. arvensis	SRS3100	0.16	31.15	1.88	10.92	0.17	7.47	0.07	6.05	1.55	0.95

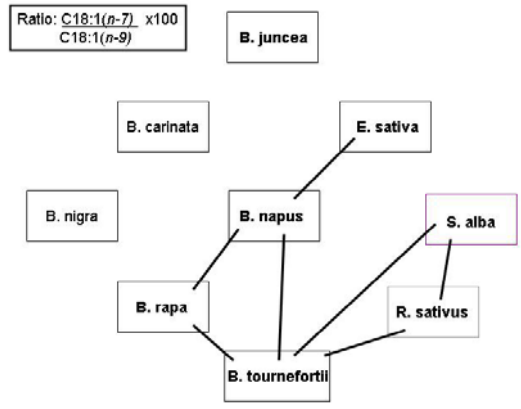

Figure 2: Chemotaxonomy of tested Brassicaceae according to the C18:1 (*n-7*)/(*n-9*) ratio.

Table 2: Ratio (*n-7*)/(*n-9*) for C18:1, C20:1 and C22:1 (as percent) for all tested *Brassica* species

Genus	Species	N	C18:1 Ratio (%)		C20:1 Ratio (%)		C22:1 Ratio (%)	
			Average	Stdev	Average	Stdev	Average	Stdev
Brassica	*carinata*	5	12.03	1.52	19.19	2.46	12.03	1.52
Brassica	*juncea*	6	7.95	1.55	9.46	7.65	7.95	1.55
Brassica	*napus*	5	6.22	1.89	5.92	5.85	6.22	1.89
Brassica	*nigra*	4	15.88	1.32	17.72	2.87	15.88	1.32
Brassica	*rapa*	5	5.51	0.87	9.30	11.53	5.51	0.87
Brassica	*tournefortii*	5	5.28	0.38	11.70	1.66	5.30	0.38
Eruca	*sativa*	5	6.69	0.66	15.80	3.0	6.69	0.67
Raphanus	*sativus*	5	4.30	0.60	4.41	1.48	4.30	0.60
Sinapis	*alba*	5	4.43	0.56	7.08	0.89	4.43	0.56
Camelina	*sativa*	1	5.54		3.18		1.04	
Cramble	*Abyssinica*	1	2.83		29.53		1.97	
Sinapis	*Arvensis*	1	6.05		1.55		0.95	

Table 3: Statistical analyse s of the ratio [(*n-7*)/(*n-9*)]x100 for C18:1, one-way analysis of variance.

	B. juncea	*B. napus*	*B. nigra*	*B. rapa*	*B. tournefortii*	*E. sativa*	*R. sativus*	*S. alba*
B. carinata	***	***	***	***	***	***	***	***
B. juncea		***	***	***	***	***	***	***
B. napus			***	NS	NS	NS	***	***
B. nigra				***	***	***	***	***
B. rapa					NS	*	*	*
B. tournefortii						**	NS	NS
E. sativa							***	***
R. sativus								NS

*** P < 0.001 ** P < 0.01 * P < 0.05 NS: P > 0.05

Changes in the contents of glucosinolates during crop development in different parts of rapeseed varieties

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Abstract

Changes in the amounts of individual glucosinolates (GSL) in Japanese rapeseed (*Brassica napus* L.) varieties were measured in the vegetative and reproductive tissues. Three zero erucic cultivars (Asakanonatane, Nanashikibu, Kizakinonatane) and one double-low cultivar (Kirariboshi) were used. The GSL contents were largely different depending on the plant parts, developing stage and cultivars. Progoitrin and gluconapin were mainly found in the seeds. Their contents were different among cultivars. In the non double-low (single-low) varieties, their contents did not change or increased to some extent with maturing. And the double-low variety, Kirariboshi, contained almost no progoitrin or gluconapin throughout its growth stages. Glucobrassicanapin, glucobrasscin and gluconasturtiin were found in all four varieties, mainly detected in their roots. Even in the double-low variety, Kirariboshi, they were detected. The content of glucobrassicin in the roots tended to decrease with maturing. And that of gluconasturtiin was similar in all cultivars at harvest time. But the patterns of its change were different among the cultivars. In Kizakinonatane, its content was decreased with maturing. In Asakanonatane, Kirariboshi and Nanashikibu, it did not change largely.These results showed that GSL contents in Japanese rape (*Brassica napus* L.) were different among plant parts, maturing stage and cultivars. And it was found that even a double-low variety contained some GSL in the roots. More information is needed for clarifying if the GSL in the roots have some effects or physiological function on the subsequent crops.

Key words: *Brassica napus* L., glucosinolate (GSL), plant parts, cultivar

Introduction

GSL are contained in parenchyma of *Brassica napus* L.. Because its hydrolysis products, thiooxazolidone and isothiocyanate were toxic (Astwood E. B. *et al*.1949, Kawagishi S.1985), low GSL varieties were bred. Regarding its hydrolysis products, the biofumigation effect to soil-borne pathogens was reported (Bellostas N. *et a*l. 2004). And Smith B. J. *et al*. (2002) reported that hydrolysis of glucosinolates in root tissues affected to growth of fungi and oomycetes.In our laboratory we are studying rotational cropping system using rape, barley and sunflower. In the field test, the growth of sunflower after rape was poorer than after barley. One of the aims of the present study was to clear the mechanisms of this phenomenon and therefore, we analyzed the contents of GSLs in stems, leaves, roots and reproductive organs at different growth stages for studying of its effects to succeeding crops.

Material and methods

Plant materials The rapeseed of "Asakanonatane (Norin No.46)", "Kizakinonatane (Norin No.47)" and "Nanashikibu (Norin No.49)" were zero erucic cultivars (single-low cultivars). And "Kirariboshi (Norin No.48)" was zero erucic and low seed-glucosinolate cultivar (double-low cultivar).*Field experiments* Field experiments were conducted in 2005 and 2006 at the experimental field station of the National Agricultural Research Center (Tsukuba, Japan) on a rotational paddy field in upland conditions. The seeds were sown in 30 × 12m plots per one cultivar. They were sown in the rows 0.3m apart. The space between plants was 5cm. Fertilizer mixture of 106-5-94 kg N, P_2O_5, K_2O, ha^{-1} was uniformly broadcast over the experimental area. Weeds were removed manually. *Sampling* Samples were taken in three replications at April 25, May 9, 23, June 6, 20 in 2005, and at April 14, 25, May 23, June 20 in 2006. They were separated to leaf, seed, pod, stem, and roots immediately after the sampling, and frozen in liquid N_2 before freeze-drying.*Glucosinolate analysis* Dry samples were milled to fine powder before GSL analysis. GSL extraction and determination were performed as previously described (Ishida *et al*. 1995) using sinigrin as an extraction standard. Separation and detection of desulphoglucosinolates were performed using a Shimazu SPD-10Avp HPLC (Shimazu, Tokyo, Japan) fitted with a 4.6 × 250mm i.d. Intersil ODS-3 (particle size 5μm) column (Shimazu, Tokyo, Japan) and the eluate was detected at 228nm by a UV detector SPD-10AVvp (Shimazu, Tokyo, Japan). Analyses were done in two or three replications for each sample. The glucosinolates were identified

Results

We defied that the major GSL, progoitrin and gluconapin, were contained mainly in seeds and pods (Table). The content of progoitorin in seeds and pods was kept high and that in other parts decreased with maturing (Fig. 1). The content of gluconapin in seeds and pods was once decreased and then increased with maturing. And in other parts it decreased with maturing. About 4-hydroxy-glucobrassicin, the content in seeds and pods was high at about harvest time. In other parts, it

didn't change and was kept low. Glucobrassicin was mostly present in the roots. Its content in roots tended to decrease with maturing. At harvest time, the contents of glucobrassicin and gluconasturtiin in roots between single- and double-low cultivars were not different significantly at the 5% error level (Table, Fig. 2.). The changing patterns of their contents were almost similar in 2005 and in 2006 (data not shown).

Discussion

The changes in the GSL concentrations during crop development were reported by Fieldsend and Milford (1994). It was reported there were differences in the GSL concentration among cultivars and plants parts (leaves, stems, buds, pods and seeds). In this paper, the contents of GSL in the roots were also analyzed. The major finding of this report was that the glucobrassicin and gluconasturtiin are mainly contained in the roots, and their contents in Kirariboshi, a double-low variety, was as high as in the single-low varieties (except gluconasturtiin in Kizakinonatane). About glucosinolates from roots, Kirkegaard *et al.* (2001) reported their biofumigation function. Rumberger and Marschner (2004) reported that rhizosphere bacterial community composition was correlated with the glucosinolate concentration in roots. Probably for these eco-physiological functions, even the double-low cultivar maintained some levels of glucosinolate contents in plant parts (including roots) other than seeds.

The glucobrassicin and gluconasturtiin in the roots could play the suppressing role on the growth of the succeeding crops even after the double-low rapeseed such as 'Kirariboshi'.

References

Astwood E. B., M. A. Greer and M. G. Ettlinger (1949) *l*-5-Vinyl-2-thiooxalidone, an antithyroid compound from yellow turnip and from brassica seeds. *J. Biol.Chem.*181: 121-130.

Bellostas N., J. C. Sorensen and H. Sorensen (2004) Qualitative and quantitative evaluation of glucosinolates in cruciferous plants during their life cycles. *Agroindustria* 3: 5-10.

Fieldsend J. K. and G. F. J. Milford (1994) Changes in glucosinolates during crop development in single- and double- low genotypes of winter oil seed rape (*Brassica napus*) : Production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within the crop. *Ann. Appl. Biol.* 124: 531-542.

Ishida M., Y. Okuyama, Y. Takahata and N. Kaizuma (1995) Varietal diversity of seed glucosinolates content and its composition in Japanese Winter Rape (*Brassica napus* L.). *Breed. Sci.* 45: 357-364.

Kirkegaard J. A., B. J. Smith and M. J. Morra (2001) Biofumigation: soil-borne pest and disease suppression by Brassica roots. *Root Research* 10 (extra issue 1): 416-417.

Kawagishi S. (1985) Glucosinolates-their enzymatic degradation, and reactivity and toxicity of degradation products. Nippon Shokuhin Kogyo Gakkaishi 32(11): 836-846.

Rumberger A. and Marschner P. (2004) 2-Phenylethylisothiocyanate concentration and bacterial community composition in the rhizosphere of field-grown canola. *Functional Plant Biology* 31: 623-631.

Smith B. J. and J. A. Kirkegaard (2002) *In vitro* inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. *Plant Pthology* 51:585-593.

Table Differences in the contents of major glucosinolates in plat parts at harvest time in 2005.

Plant parts	Cultivars	Progoitrin	Gluconapin	4-Hydroxy-Glucobrassicin	Glucobrassicanapin	Glucobrassicin	Gluconasturtiin
		μ mol/g D.W.					
Seeds&Pods	Kizakinonatane	12.37±0.03**	6.12±0.6**	0.22±0.03	n.d.	0.03±0.01	n.d.
	Kirariboshi	0.17±0.00	0.09±0.0	0.65±0.13*	0.59±0.09**	n.d.	n.d.
Stems	Kizakinonatane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Kirariboshi	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Roots	Kizakinonatane	0.31±0.12	0.14±0.03*	n.d.	n.d.	0.67±0.08	0.38±0.03
	Kirariboshi	n.d.	n.d.	n.d.	0.34±0.12	1.50±0.66	0.34±0.00

Means±SD and represented as relative values to sinigrin (internal standard). n.d.=not detected. Means of about three replicates±S.E. of the mean.

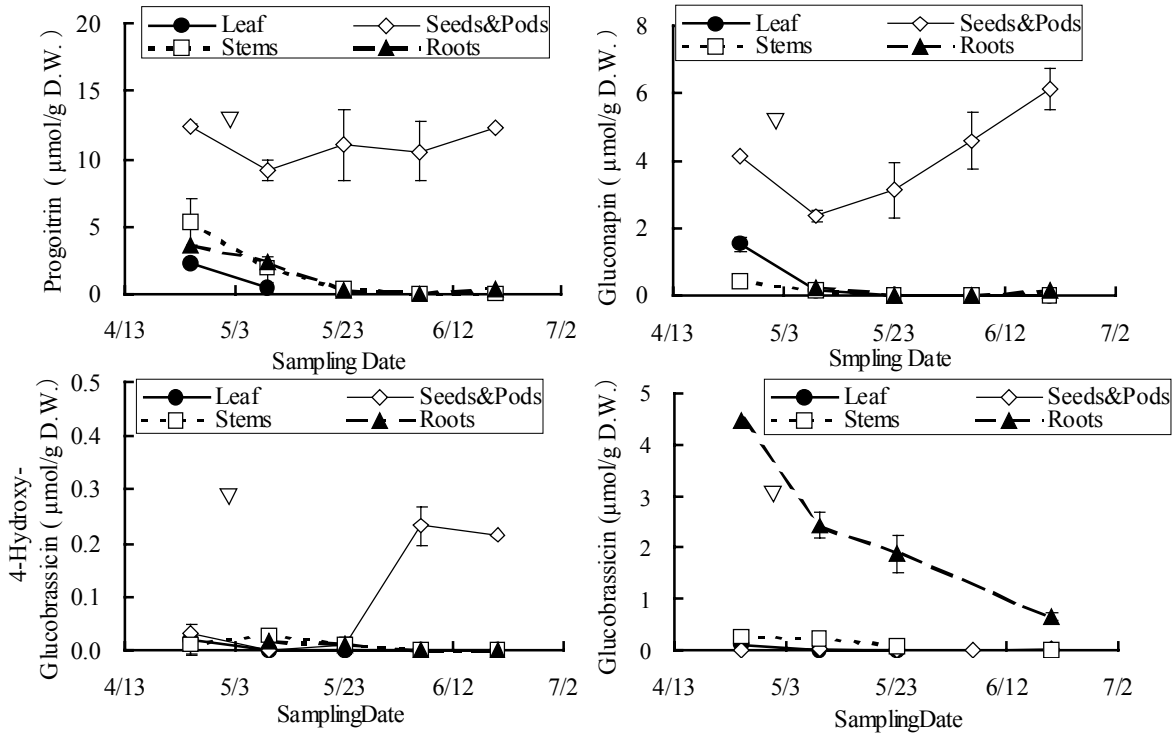

Fig. 1. Changes in the contents of major glucosinolate in different plat parts in 2005 (Kizakinonatane).
Means of about three replicates ± S.E. of the mean. ▽ Termination of flowering

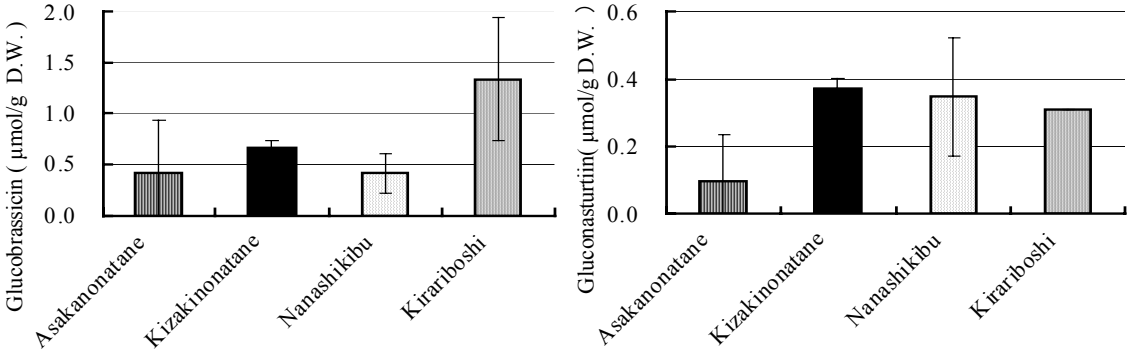

Fig. 2. The contents of glucosinolates (glucobrassicin and gluconasturtiin) in roots at harvest time in 2005.
Means of about three replicates ± S.E.of the mean.

An effective and fast way to publish facts and figures from variety trials to farmers

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Abstract

In order to make it possible for the farmer to grow the best varieties a quick and efficient presentation of new results from field trials is important. In Denmark all results and scores from variety trials are published on www.sortinfo.dk. The website is in Danish and English. Only a couple of days after registration in the field all scores from the variety trials are available on the internet. The site shows the varieties that have been tested, yields, winter hardiness, agronomic data, resistance to pests and diseases, agronomic characteristics, quality, etc. Data and scores from one year or more years can be studied, as well as data from single locations and on average can be seen.

The system and the website Sortinfo.dk has been developed in cooperation between the Official variety testing and National field trials in Denmark. The field trial system is integrated, and the guidelines for scores and registrations are the same. In this way all data from the variety trials can be published in a simple and easy way, and the knowledge can be used by advisers, farmers and breeders right a way. The benefit for the farmer is that he has access to independent information which enables him to grow the best varieties, taking the specific conditions for his property in consideration. It is possible to develop this system and gather all scores and figures related to the varieties from variety trials all over Europe or the whole world. To make this possible a uniform guideline for running and scoring variety trials is needed. In addition, a common database and a homepage must be developed. The benefits are more knowledge about the varieties, which will provide a more stable production, and the opportunity for integrated pest management.

Introduction

The cultivated area in Denmark is approx. 2,500,000 ha. 180-200,000 ha are grown with winter oil seed rape in 2007. Five to ten varieties are grown with the most dominant covering approx. 40 pct. of the area. The area with winter wheat, winter barley, winter triticale and winter rye is approx. 850,000 ha and the area with spring seed is approx. 650,000 ha. The areas with grass and other fodder crops is approx. 610,000 ha.

In order to optimize the economic yield and minimize the use of pesticides it is important to have good knowledge about the characteristics of the varieties. In Denmark variety trials are conducted each year in almost all annual crops, where crop yields as well as cultivation characteristics and resistance to diseases are registered. The results from these trials are published currently on www.sortinfo.dk. Danish farmers choose varieties on the basis of the results of these trials.

Application for variety trials

Plant breeders and variety representatives apply for varieties that they want to have tested in the variety trials.

The breeers and representatives pay a fee that covers the costs for the variety trials.

The trials are conducted in cooperation between the Danish Plant Directorate, dept. of variety testing, at Tystofte, and the Danish Agricultural Advisory Service, National Centre, Crop Production. Each year trials in winter oil seed rape are conducted on 8 localities. In recent years about 100 winter oil seed rape varieties have been tested each year.

Planning of trials

Varieties, of which tests are wanted, must be entered for trials and seed must be delivered before 10 August. A trial plan is made for the variety test using an Alpha design with three replications. The seed is weighed out, 65 viable seeds are sown per m^2 in hybrid varieties and 80 viable seeds per m^2 in line varieties. In Denmark the optimal time of establishing winter oil seed rape is 15-20 August.

The varieties are sown in "plot in plot" with two rows of the same variety on both sides of the net plot. This is in order to avoid neighbouring effects. The net plot is 12 m^2.

Assessment of the trial

In the trial plan the conditions under which the trial should be established are indicated, which treatments to make during the growing season, which registrations and scores to make, how to harvest the trial plot, how to take samples and where to send the samples. The trial plan also includes which analyses to conduct. In order to ensure uniform assessments in all trials, guidelines are made for how to make assessments and how to make analyses. The guidelines can be studied at www.lr.dk – Nordic Field Trial System.

Data collection

When the trial plan has been made in the Nordic Field Trial System, the plan is transferred to PC field trial. From here the plans can be transferred to PDA's that can be taken to the trial field, so that all registrations can be entered. From the PDA all data can be transferred to the Nordic Field Trial System. Harvest and analysis results are collected and transferred in the same way to the Nordic Field Trial System.

Photo 1. "Plot in plot". In the middle six rows, net plot. The two rows on each side outside the plot borders are the same varieties.

PlanteInfo: SortInfo - Microsoft Internet Explorer

Filer Rediger Vis Foretrukne Funktioner Hjælp

Tilbage Søg Foretrukne

Adresse http://www.sortinfo.dk/Oversigt.asp Gå Hyperlinks

Google Go Bookmarks 35 blocked Check AutoLink AutoFill Send to Settings

SortInfo 24 November 2006 Pl@nteInfo

Select crop: Winter rape SortsValg Download Find variety Select varieties Select info Help

Overview for winter rape (varieties in National trials 2006, latest data)

		Yield 1)		Agronomic data 1)			Cultivation 2)	Variety charactersitcs 8)	Quality 3)
		Seed yield std.quality. Indeks (index)	Seed yield std.quality Tested variety (hkg/ha)	Pct.oil in dry matter Tested variety (% dry matter)	Cropheight at harwest, Tested variety (cm)	Tendency to lodging Tested variety (score 0 - 10)	Winterhardiness (score 0 - 10)	Type of Variety	Content of glucosinlates (mikromold/g seed)
Year		2006	2006	2006	2006	2006	2003	2006	2006
Sorting									
1.	Bandit	107 (8)	4.366 (8)	50,0 (8)	150 (7)	1,3 (7)		Hybrid	
2.	PR46W14	106 (8)	4.341 (8)	49,5 (8)	146 (7)	1,6 (7)		Hybrid	
3.	Casoar	106 (8)	4.332 (8)	48,6 (8)	134 (7)	1,2 (7)			12
4.	Catana	106 (8)	4.330 (8)	50,8 (8)	140 (7)	1,7 (7)			
5.	Labrador	106 (8)	4.320 (8)	48,4 (8)	130 (7)	1,8 (7)	8,7 (2)		8
6.	Vision	106 (8)	4.318 (8)	49,6 (8)	150 (7)	0,6 (7)		Population	
7.	Excalibur	105 (8)	4.289 (8)	49,0 (8)	145 (7)	1,3 (7)		Hybrid	20
8.	Catalina	105 (8)	4.282 (8)	49,0 (8)	134 (7)	1,6 (7)	8,9 (2)		16
9.	PR46W09	105 (8)	4.275 (8)	48,9 (8)	148 (7)	1,3 (7)		Hybrid	7
10.	Lorenz	105 (8)	4.274 (8)	50,8 (8)	149 (7)	0,6 (7)			
11.	ES Astrid	104 (8)	4.245 (8)	48,0 (8)	132 (7)	0,3 (7)			12
12.	PR46W31	103 (8)	4.229 (8)	48,2 (8)	157 (7)	1,5 (7)		Hybrid	13

Internettet

start Indbakke - Microsoft ... crh20061121Kina_DK... PlanteInfo: SortInfo -... 10:37

Figure 1. Screen dump from SortInfo.dk with selected characteristics.

Dissemination of knowledge

SortInfo is an online portal accessible for all who are interested. www.sortinfo.dk has been developed for all engaged in cultivation of oilseed rape and other crops in order to provide access to all registered information about varieties that are on the market or are requested on the Danish market. SortInfo is updated regularly, and new registrations are available few days after having been assessed or measured in the trials. As for winter oil seed rape data are available on variety yields, oil content, winter hardiness, flowering time, plant height, disease resistance, crop height at harvest, tendency to lodging, quality, variety type etc. In SortInfo it is possible to find the actual as well as historical data.

SortInfo is available in Danish as well as in English, enabling both Danish and foreign farmers, breeders, variety representatives and others interested to find the results from the trials.

Number of visitors on SortInfo.dk

In recent years approx. 50,000 hits have been made on SortInfo.dk, with the majority in August, when the new harvest results are published. But throughout the year there are between 500 and 1,500 hits per week. SortInfo is visited by farmers, advisers, variety owners, variety representatives, and others searching for information on specific varieties.

SortInfo – EuroVarietyInfo or WorldVarietyInfo

In order to be able to grow the best varieties, with high yields and the best resistance to diseases a SortInfo for the whole of Europe or for the whole world would be a good approach. By having access to as much information as possible on cultivation of the varieties, resistance and quality properties, it would be possible to chose and grow varieties with a minimum use of pesticides. This would benefit the growers of oilseed rape who would be able to reduce the use of pesticides, thereby reducing the costs for the environment by avoiding unnecessary effects from use of pesticides.

In order to develop a joint EuroVarietyInfo or WorldVarietyInfo development of common guidelines for conducting, assessments and registrations of the trials would be necessary. If assessments are conducted according to joint guidelines, it would be possible to publish the results in a program such as SortInfo.

Inserts for FOSS NIRS 6500 spinning ring cups

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Abstract

Near infrared reflectance (NIR) analysis has proven be a very efficient tool for elevating seed quality for rapeseed and mustard breeders. Calibrations for oil, protein, fatty acids, glucosinolate, fibre, chlorophyll, etc. are routinely utilized by many laboratories supporting rapeseed breeding efforts around the world. The FOSS NIRS 6500 instrument is a popular model. With the use of an autoloader and the spinning ring cup attachment the analysis is simple and rapid. Intact seed can be utilized which makes it a non-destructive method. The spinning ring cup holds about 4-5 grams of seed and is therefore useful for examining samples harvested from large or small plots and individual 3 meter rows. However the minimum sample size of about 4 grams limits its usefulness for samples from individual plants harvested from field plots or from the greenhouse. We found the inserts sold by FOSS for these spinning ring cups impractical and too expensive for routine use, so we designed our own. A local machine shop made three sizes for us with the following nominal sizes: two gram, one gram and 0.5 gram (Cost: $6.00 each). The inserts allow intact seed samples from individual plants to be analyzed by the FOSS NIR instrument. The insert size choice depends on the size of the smallest samples.

Key words: NIR, sample cup inserts, single plant analysis, oilseeds

Introduction

NIR is widely accepted by oilseed breeders and chemists, replacing several traditional methods of analysis. NIR is used to predict oil, protein, glucosinolate (GSL), fatty acids, fiber and chlorophyll on intact seed samples of rapeseed and mustard (Biston et al. 1987, Daun et al. 1994, Font et al. 2003, Renard et al. 1987, Sato et al. 1998, Velasco et al. 1998, Williams and Sobering 1993, and others). The FOSS NIRS 6500 instrument is popular and its spinning cup attachment allows for simple, rapid analysis. Individual plants harvested from the field or greenhouse often yield an amount of seed which is insufficient to be analyzed in the full size cups. In this paper we describe inserts which can used to allow analysis of these plants.

Material and Methods

Cup Inserts

Inserts were designed for standard sample cups utilized by the autoloader attachment of our NIR (NIRS system model 6500, FOSS NIRSystems, Silver Springs, MD, USA). Aluminum inserts rings were designed to exactly fit the inside of the sample cups. They were 38 mm in diameter by 9 mm thick with an inside diameter of 13 mm, 20 mm and 25 mm, to accommodate approximately 0.5 gram, 1 gram or 2 grams of seed respectively. A machine shop (Nutana Machine Ltd., 2615 1st Ave. N, Saskatoon, SK, Canada, Fax: 1-306-242-2671) manufactured 100 of each size (see Fig. 1). The inserts are held in place using a strip (25 mm by 6 mm) of black foam window insulation attached to the side of the insert. The backings for the inserts are 13, 20, and 25 mm Tegrabond septa (Chromatographic Specialties Ltd.). The inserts are easily removed from the cups when not needed.

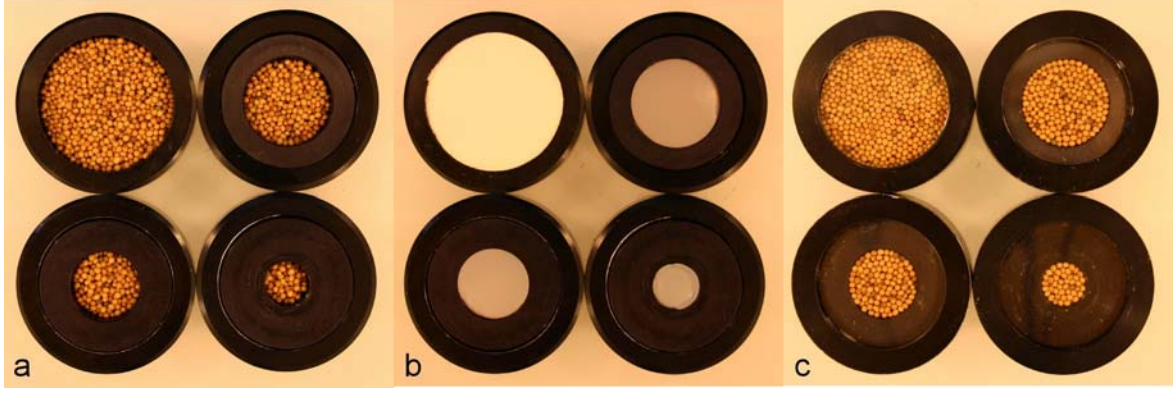

Figure 1: a) inserts filled with seed. b) inserts with backing on. c) inserts front view. Upper left: original cup, Upper right: 2 gram insert. Lower left: 1 gram insert. Lower right: 0.5 gram insert.

Seed Material and NIRS analysis

Seed samples used for calibration of the NIRS instrument were selected from seven oilseed species that normally enter

our laboratory for analysis including: *Brassica napus, Brassica juncea, Brassica rapa, Brassica carinata, Sinapis alba, Camelina sativa and Linum usitatissimum.* Two sets of samples were created, one for calibration (1365 samples) and a separate set for validation (272). Intact seed samples, dried at 40°, were in placed in the cups, scanned and the entire spectral range of the instrument collected. The same seed samples were scanned again using the insert rings and spectra collected. Equation generation and data transformation were done using WINISI III (version 1.50e) software. The modified partial least squares (MPLS) regression method was chosen as well as scatter and detrend correction. All wavelengths were utilized and a second derivative mathematical treatment (2,4,4,1) was applied. Calibrations were created for oil, protein and insoluble fibre content, seed color, individual fatty acids and GSL contents, both total and individual.

Reference Methods

Oil content, protein content, fibre content, seed color, fatty acid composition and GSL content were determined by reference methods available in the laboratory. Oil content was estimated on 20-25 g intact seed samples with a pulsed NMR instrument (Bruker Minispec, 10 MHz magnet, 40 mm probe assembly, Bruker Optics Ltd, Milton, Ontario, Canada), calibrated according to manufacturer's instruction and corrected using standards for each species. Protein content was determined with an LECO FP-428 on 0.5 gram intact seed samples. Acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) contents were measured with an ANKOM^{200} Fiber analyzer (ANKOM Technology, Macedon, NY, USA). The manufacturer's standard procedures were followed. Seed color (whiteness index, WI) was determined with a HunterLab Miniscan colorimeter. Oil, protein and fibre contents are reported as percent of dry seed.

Seed fatty acid composition was determined by gas chromatography (GC). Three gram seed samples were ground with 14 mm stainless steel balls in 4 ml hexane in 20 ml PET scintillation vials (Wheaton) placed in an Eberbach reciprocating shaker for 1 hour, after which a 10 µl aliquot was placed in a GC autosampler vial containing 50 µl hexane and treated with 100 µl 0.8% metallic sodium in methanol (Thies, 1971). After 15 minutes 50 µl 0.2 M $NaPO_4$ pH 7 was added, methanol and hexane evaporated under a stream of air (1 minute), and then 0.5 ml of heptane was added. The samples were injected into an FID equipped GC (model 6890 Agilent Technologies, Santa Clara, CA, USA; column: HP-Innowax, 7.5 m × 0.25 mm × 0.5 µm, hydrogen, constant flow, 1.3 ml/min; injector: 280°, 1 µl, split 1:40; oven: 190 - 240°, 20°/min., final time 0.6 min.; detector 300°). Oleic (18:1), linoleic (18:2), linolenic (18:3) and erucic (22:1) are expressed as percentages of all fatty acids detected. Two additional parameters are calculated, oil hydrogen density (HD) and iodine value (IV).

The GSL content of the seed was determined by GC of trimethylsilylated derivatives of desulphated GSLs by a modification of the method of Thies (1976). Samples (1 g) were ground with steel balls in a mixture of 5 ml methanol, 2 ml 1 mM benzyl GSL, 0.2 ml barium/lead acetate in 20 ml PET vials, then placed in a reciprocating shaker for 1 hour. After centrifugation 1 ml was pipetted onto 0.125 ml DEAE-Sephadex A-25 in Bio-Rad microcolumns. The columns were washed with 70% methanol, 6% acetic acid, water and 0.02 M pyridine actetate pH 5.8. Then 0.05 ml of arylsulfatase (type H-1, *Helix pomatia*, Sigma Aldrich) was added. After overnight incubation the desulpho-GSLs were eluted with water, evaporated to dryness at 60° under a stream of air, derivatized with 0.3 ml silylation reagent (pyridine: N,O-bis(trimethylsilyl)-acetamide: chlorotrimethylsilane, 18:10:1) and injected into an FID equipped GC (column: DB-1, 15 m × 0.25 mm × 1.0 µm, hydrogen, constant flow, 1.3 ml/min.; injector: 270°, 1 µl, split 1:30; oven: 260 - 320°, 20°/min, final time 0.8 min.; detector 340°). Individual and total GSLs are expressed in µmoles/gm seed.

Table 1: Summary of reference method data for calibration and validation sample sets

Constituent	Calibration Set						Validation Set					
	N	Mean	Min.	Max.	StD	Rge	N	Mean	Min.	Max.	StD	Rge
Oil	1057	42.1	24.8	52.9	5.3	28.1	212	41.7	25.9	52.5	5.4	26.6
Protein	1350	28.5	14.9	42.7	4.0	27.8	271	28.8	18.9	40.8	3.9	21.9
NDF	460	16.7	9.5	25.0	2.6	15.4	91	16.3	10.4	23.6	2.9	13.2
ADF	456	11.2	5.1	20.8	2.7	15.7	91	10.7	5.6	18.6	2.6	13.0
ADL	460	2.7	0.3	9.4	1.8	9.2	91	2.4	0.4	7.7	1.7	7.3
18:1	707	44.2	6.2	76.5	22.1	70.3	139	44.1	6.1	73.9	21.7	67.8
18:2	707	19.9	7.9	75.6	5.3	67.7	139	21.2	8.9	74.6	8.8	65.7
18:3	707	12.9	1.3	69.4	11.1	68.0	139	12.5	1.8	62.6	10.9	60.8
22:1	707	9.4	0.0	55.3	16.1	55.3	139	9.6	0.0	55.9	16.6	55.9
IV	707	119.5	92.2	210.5	19.3	118.3	139	120.0	96.7	201.1	19.2	104.4
HD	707	11.64	10.97	11.97	0.16	1.00	139	11.64	11.03	11.95	0.16	0.92
Sinigrin	598	56.1	0.0	169.3	59.0	169.3	121	54.2	0.0	167.2	58.9	167.2
Sinalbin	598	6.0	0.0	194.7	30.1	194.7	121	8.2	0.0	204.9	36.2	204.9
aGSL	598	67.2	0.1	170.2	56.0	170.1	121	65.6	0.0	168.1	56.1	168.1
tGSL	598	79.7	1.7	200.5	56.0	198.8	121	78.8	0.8	212.0	57.6	211.3
WI	943	-17.6	-42.7	2.8	12.9	45.4	184	-19.0	-41.2	2.2	12.8	43.4

* N – number of samples with reference method data; StD – standard deviation; Rge – range of values found; aGSL – total aliphatic glucosinolates; tGSL – total glucosinolate content

Results

Reference data is shown in Table 1. For all constituents a broad range of values is observed, covering the expected ranges to be seen at our laboratory. The NIRS statistics of the second derivative equations for the full cup and 1 gram size are shown in Table 2. A good correlation between the NIRS prediction and reference data is found for most of the constituents for both

the standard cup and the reduced sample size using the 1 gram inserts. Generally there is little or no decline in NIR prediction efficiency between the full size cup and the inserts as estimated by RSQ and SEP values. The bias and slope of the equations are also mostly acceptable. The RPD and RER values (Williams and Sobering, 1993) for oil, protein, 18:1, 18:3, IV, HD, GSLs and seed colour indicate that the NIR predictions either with full size cup or the 1 gram insert may be used as a selection tool for breeders. Of the fatty predictions, HD and IV are the strongest. Calibrations for 18:2 and the insoluble fibre constituents are less impressive, but ADL is certainly useful for breeding purposes.

Table 2: NIR validation set statistics for the second derivative equations for the full size cups and 1 gram inserts

Constituent	Full size cups						1 gram inserts					
	SEP	Bias	Slope	RSQ	RER	RPD	SEP	Bias	Slope	RSQ	RER	RPD
Oil	0.8	0.06	1.01	0.980	34.6	7.0	0.7	-0.03	1.00	0.982	36.8	7.5
Protein	0.6	0.02	1.00	0.980	39.6	7.1	0.6	0.08	1.00	0.976	36.2	6.4
NDF	1.2	0.19	1.05	0.820	10.8	2.3	1.4	0.16	1.11	0.784	9.8	2.1
ADF	1.1	0.06	0.99	0.817	11.8	2.3	1.1	0.05	0.97	0.819	11.8	2.4
ADL	0.4	0.02	1.02	0.934	16.3	3.9	0.4	0.02	0.99	0.942	17.4	4.2
18:1	2.8	-0.04	1.02	0.983	24.1	7.7	3.6	-0.16	1.00	0.973	18.9	6.1
18:2	2.9	0.55	1.24	0.929	22.7	3.0	2.5	0.33	1.13	0.934	26.6	3.6
18:3	1.1	-0.07	1.03	0.990	55.0	9.9	1.5	-0.04	1.02	0.982	41.2	7.4
22:1	2.6	0.01	1.03	0.976	21.3	6.3	2.6	0.13	1.00	0.976	21.9	6.5
IV	1.4	0.10	1.01	0.995	73.7	13.5	1.9	0.16	1.01	0.991	56.2	10.3
HD	0.01	0.00	1.03	0.993	65.9	11.5	0.02	0.00	1.01	0.989	54.3	9.5
Sinigrin	7.9	-1.07	0.98	0.982	21.1	7.4	8.4	-0.25	0.98	0.980	20.0	7.0
Sinalbin	9.3	-0.53	1.00	0.934	22.1	3.9	7.1	-0.73	0.98	0.963	29.1	5.1
aGSL	4.8	-0.36	0.99	0.993	35.2	11.7	4.0	-0.24	0.99	0.995	41.6	13.9
tGSL	10.4	-0.94	0.98	0.968	20.4	5.6	7.6	-1.42	0.99	0.983	27.6	7.5
WI	1.9	-0.13	1.01	0.977	22.3	6.6	1.8	0.01	1.01	0.981	24.4	7.2

* RSQ- coefficient of determination; SECV – standard error of cross validation; SEP – standard error of prediction; RPD –StD of validation / SEP (Williams and Sobering, 1993); RER –range of validation / SEP

Table 3: Use of NIR fatty acid 1 gram prediction in a F_2 plant population segregating for 18:1 and 18:3

Constituent	GC Data					NIR Prediction Statistics					
	N	Mean	Min.	Max.	Rge	StD	SEP	Bias	Slope	RSQ	RER	RPD
18:1	100	72.0	57.0	85.1	28.1	8.0	3.4	-0.68	0.87	0.845	8.3	2.4
18:2	100	14.7	2.8	26.0	23.2	7.7	2.1	-0.46	1.01	0.926	10.9	3.6
18:3	100	4.6	2.1	10.0	7.9	1.7	1.6	0.92	0.66	0.605	5.1	1.1
IV	100	101.6	87.4	119.0	31.6	7.6	2.1	1.23	0.90	0.960	15.1	3.6
HD	100	11.73	11.60	11.83	0.22	0.05	0.02	-0.01	0.90	0.951	14.9	3.5

As a test for the ability of the inserts to be used for fatty acid selection 940 F_2 plants of three breeding populations of *Brassica napus* canola, segregating for 18:1 and 18:3 and seed color, were processed by the NIR with the 1 gram inserts. These populations were unrelated to the samples that were used for calibration and validation sets and the plants were pre-selected for yellow seed color. 100 plants were chosen which represented the entire range of HD and IV values predicted by the NIR and included 48 considered to be candidates for advancement. These plants were analyzed for fatty acid composition by GC. In this case the sample size was 30 seeds to avoid destruction of valuable seed as much as possible. The comparison of NIR prediction values and reference method data is shown in Table 3. Because of the small sample size used for GC analysis it is to be expected that the correlation would be affected due to sampling error, but nevertheless the SEP values of all fatty acids are similar to the original validation set SEP values. There is a strong correlation of the NIR values for IV (RSQ: 0.960) and HD (RSQ: 0.951) and RER and RPD values indicate that these two can be selected for effectively by NIR. Most of the plants selected by NIR using HD and IV for advancement were also selected by GC for advancement using the complete fatty acid information valuable. Using the NIR eliminated the need to do GC analysis on all 940 plants. The correlation between NIR and GC for the individual fatty acids 18:1, 18:2 and 18:3 is less strong, but RER and RPD statistics suggest that NIR predictions based on them might also be useful.

Discussion

Under western Canadian conditions it is a frequent occurrence that individual plants will yield less than five grams of seed. For example, of 1600 plants harvested from a yellow mustard 2006 isolation only approximately 400 yielded 4 grams of seed or more meaning that probably half of the plants could not processed by the FOSS NIR using the full size cups, however with the 1 gram inserts over 90% can be. Others have reported the construction of small size cups (Sato et al. 1998) and inserts (Velasco et al. 1999) which have been used for the single seed analysis, but the inserts described here fit our analytical needs for single plant analysis. The fact that 1 gram inserts are seen to have little negative impact of the NIR predictions is probably a result of duplicating the material (anodized aluminum) used in the original full size sample cups.

Conclusions

The 1 gram inserts created for the FOSS NIRS 6500 sample cups have little negative impact on the efficacy of NIR predictions for oil, protein, fatty acids, glucosinolates, seed color and fibre. They are easy to use, inexpensive and provide breeders access to non-destructive NIR predictions for samples with limited amounts of seed (seed harvested from individual plants). The results for the 0.5 gram and 2 gram inserts are not reported here, but it can be expected that the results with them would be similar.

References

Biston R., Dardenne P., Cwikowski M., Wathelet J-P., Severin M. (1987). Analysis of quality parameters of whole rapeseed by N.I.R.S. World Crops: Production Utilization and Description **13**, 163-172.

Daun J.K., Clear K.M., Williams, P. (1994). Comparison of three whole seed near-infrared analyzers for measuring quality components of canola seed. J. Am. Oil Chem. Soc. **71**, 1063-1068.

Font R., del Rio M., Fernandez J.M., de Haro A. (2003). Acid detergent fiber analysis in oilseed *Brassicas* by near-infrared spectroscopy. J. Agric. Food Chem. **51**, 2917-2922.

Renard M., Bernard C., Deschamps M., Furtoss V., Lila M. Quinsac A., Regnier J.M., Ribaillier D. (1987). Glucosinolate analysis in whole rapeseed by near infrared reflectance spectroscopy. World Crops : Production, Utilization and Description **13**, 173-176.

Sato T., Uezono I., Morishita T., Tetsuka T. (1998). Nondestructive estimation of fatty acid composition in seeds of Brassica napus L. by near-infrared spectroscopy. J. Am. Oil Chem. Soc. **75**, 1877-1881.

Thies W. (1971). Schnelle und einfache Analysen der Fettsäurezusammensetzung in einzelnen Raps-Kotyledonen I. Gaschromatographische und Papierchromatographische Methoden, Z. Pflanzenzüchtg. **65**, 181–202.

Thies W. (1976). Quantitative gas liquid chromatography of glucosinolates on a microliter scale. Fette Seifen Anstrich. **78**, 231-234.

Velasco L., Schierholt A., Becker H.C. (1998). Performance of near-infrared reflectance spectroscopy (NIRS) in routine analysis of C18 unsaturated fatty acids in intact rapeseed. Fett/Lipid 100, 44-48.

Velasco L., Mollers C., Becker H.C. (1999) Estimation of seed weight, oil content and fatty acid composition in intact single seeds of rapeseed (*Brassica napus* L.) by near-infrared reflectance spectroscopy. Euphytica **106**, 79-85.

Williams P. C., Sobering D.C., (1993). Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. J. Near Infrared Spectrosc. **1**, 25-32.

Antitumor and immunomodulating activities of rapeseed polyphenols

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Abstract

The polyphenol of rapeseed shell was isolated and purified. The effects of the rapeseed polyphenol L-0, I, and II on the cellular immune response of Sarcoma 180-bearing mice were investigated. Mice were treated with three doses of the polyphenol L-0 (50, 100, and 200 mg/kg body weight), rapeseed polyphenol L-0, I (50 mg/kg body weight), and rapeseed polyphenol II (50 mg/kg body weight) for 10 days. It is studied from the aspects of tumor weight, pathology of tumor tissues, relative spleen and thymus weight, delayed-type hypersensitivity (DTH) response, phagocytosis of macrophage, Splenocyte antibody formation, lactic dehydrogenase (LDH) activity, Hemolysin in serum, and catalase activity. For the dose of the polyphenol L-0 (50, 100, and 200 mg/kg body weight), rapeseed polyphenol L-0, I(50 mg/kg body weight), or rapeseed polyphenol II(50 mg/kg body weight), a significant increase ($p<0.05$ =in relative spleen and thymus weight, DTH, and phagocytosis of macrophage was observed, and a significant decrease in tumor formation, as well as a dosage effect of rapeseed polyphenol L-0 on tumor weight was also obtained. The results showed that rapeseed polyphenol exhibited significant antitumor and immunomodulating activity on mice-transplanted sarcoma 180.

Key words: Rapeseed meal; Polyphenol;Antitumor; Immunomodulation;Sarcoma 180

Introduction

Phyto-polyphenol is a kind of polyphenol with molecular weight ranged from 500 to 3000. Phyto-polyphenol shows a lot of physiological activities, such as anti-inflammatory, bacteriostasis, anti-oxidation, anti-mutation, and many other activities due to its combination with protein and radical cleanup [1-4]. Recently more and more attention was paid to the biological activities of phyto-polyphenol in medicine.

There is a lot of poly-phenol in rapeseed meal. However, rapeseed meal was usually used as feedstuff and fertilizer. Some bioactive compounds, such as rapeseed protein, poly-phenol, polysaccharide and phytin were isolated and purified to increase the value of rapeseed meal [5]. Rapeseed polyphenol exists as the forms of phenolic acid and tannin. It has been reported that rapeseed phenolic acid exhibites as the same anti-oxidative activity as the tertbutyhydroquinone, and higher anti-oxidative activity than the mixture of butylated hydroxyanisol, butylated hydroxytoluene, and monoglyceride citrate [6].

The anti-oxidation and mechanism of rapeseed polyphenol were studied in some papers, in which results showed that rapeseed polyphenol was a good scavenger of reactive oxygen species (ROS), and inhibitor of lipoxygenase in vitro and in vivo. The mechanism of rapeseed polyphenol to inhibit oxidation is probably due to the capabilities of high de-oxidation and inhibiting the enzymes associated with oxidation [7-8].

The main purpose of this paper is to research the antitumor and immunomodulating activities of rapeseed polyphenol from the aspects of tumor weight, pathology of tumor tissues, relative spleen and thymus weight, delayed-type hypersensitivity response, phagocytosis of macrophage, Splenocyte antibody formation, lactic dehydrogenase activity, Hemolysin in serum, and catalase activity.

Materials and methods

Preparation and purification of polyphenol. The cold squeezed Huaza No.4 rapeseed meal was used (Wuhan city, China). Polyphenol was extracted from the rapeseed meal sealed in a container in organic solvent A and water (65%v/v) with 1.249 × l0 -2g/ml adjunct B at 51°C for 40min. After cooled to room temperature, the extract solution was filtered to remove the meal. Organic solvent A was removed from the filtrate in a vacuum rotary evaporator below 40°C. The aqueous solution was extracted in equal volume petroleum ether. The ether phase was freeze-dried. It was applied to Special No.1 Macro reticular Resin. After washed with distilled water to remove polysaccharide and protein impurity, it was washed with 74% ethanol aqueous solution with 0.1mol/l HCl 0.5%(v/v). The ethanol solution was collected and evaporated to remove ethanol below 40°C. After freezing, its corresponding fraction was white powder(rude polyphenol).It was then applied to Sephadex LH-20 to purify the polyphenol and washed with water, methanol-water(1:2v/v) and methanol-water(1:1v/v) successively, the corresponding polyphenol L-0, polyphenol L -I and polyphenol L -IIwas obtained.

Animals. Male and female Kunming mice (18-20g, 7-9 weeks old) were purchased from the Animal Research Center, Institute of Disease Control and Prevention of Hubei Province. The mice were housed under standard laboratory conditions, and fed with standard mouse-food pellets and water ad libitum.

Treatment of mice with Sarcoma 180 cells. Sarcoma 180 cells (purchased from Tongji Medical College,

Huazhong University of Science and Technology, Wuhan) were passed into mice ascites. Then, ascites was inoculated subcutaneously 0.2 ml (1 × 106 cells) into the right axilla of each mouse.

Normal control mice were not inoculated Sarcoma180 (group I). The mice inoculated Sarcoma 180 was divided into nine groups (group II–VIII). The mice were treated as following: (Group I) normal control, received normal saline; (Group II) model control, received normal saline; (Group III), the polyphenol L-0 (50 mg/kg body weight); (Group IV), polyphenol L-0 (100 mg/kg body weight); (Group V), polyphenol L-0 (200 mg/kg body weight); (Group VI), polyphenol L -I(50 mg/kg body weight); (Group VII), polyphenol L -II(50 mg./kg body weight); (Group VIII), positive control, received Cyclophosphamide (Cy, 20 mg/kg body weight).

Normal and model control mice received the saline intraperitoneally (i.p.), while positive control mice cyclophosphamide. The rapeseed polyphenols was dissolved in saline and was administered (i.p.) for 10 days. The dose volume was 0.2 ml.

Measurements of tumor weight, relative thymus, spleen, and liver weight. After administration for 10 days, mice were sacrificed by cervical dislocation. Spleen, thymus, and tumor weights in the mice were measured [9].

Lactic dehydrogenase (LDH) activity assay. LDH activity was measured spectrophotometrically. 3.0 ml of reaction mixtures contained 6.5 mmol/l NAD, 100mmol/l Tris HCl and 100mmol/l KCl, were added into 100μl mice serum and kept at 30°C for 30s. The absorbance of the mixture was noted at 340 nm for 2 min at regular intervals, the change of absorbance per minute (ΔA/min) is calculated. The unit activity of LDH was expressed as 1μmol /min of NADH. Total LDH activity is as the following equation [10].

$$\text{LDH(U/mL)}=4.984\Delta A/\text{min} \quad (1)$$

Catalase activity assay. The catalase (CAT) activity was measured spectrophotometrically at 230 nm. The blood from mice eyepit, after anticoagulation, centrifugation and wash with physiological saline, the erythrocytes were obtained and diluted 100 times with distilled water. 4.0ml 0.3% hydrogen peroxide was added into 6ml 50mmol/l phosphate buffer (pH 7.0),10μl erythrocyte solution was added into 3.0ml of the buffer at 25°C. The mixtures were used to measure the catalase activity. The CAT activity was measured by H_2O_2 consuming as described [11].

Phagocytosis of macrophage assay. Chicken red blood cells (CRBC) were used to assess the phagocytosis of macrophage [12-13]. Briefly, mice were sacrificed 30 min after intraperitoneally injected 1 ml 1% CRBC, and then injected 2.5 ml Hank's. Activated Macrophages were obtained by lavage of the cavity into microscope slide. After centrifugation at 150 × g for 10 min, the supernatant was removed and the free CRBC were lysed by sterile 0.16 mmol/l NH_4Cl lysing buffer. Macrophages were dyed within 0.5–1.0 min right after the microscope slide was air-dried. Microscope slide were washed by PBS and counted with microscope. The phagocytosis index was measured by counting the number of phagocytosed CRBC per 100 macrophage cells [14].

Delayed-type hypersensitivity reaction to dinitrofluorobenzene. Mice were sensitized to dinitrofluorobenzene (DNFB) by placing 25 μl 1% DNFB in acetone–gingili oil on the shaved abdominal skin of recipients on the third and fourth day. Five days later, 10 μl 1% DNFB solution was placed on the right ear. Twenty four hours later, the antigen challenge was evaluated by measuring weight difference of right and left ear with an analytical balance [15].

Splenocyte antibody assay. Splenocytes taken from mice after administration of 0.2ml 20% sheep red blood cells (SRBC) were suspended in phosphate buffer, 1.0ml 0.4% SRBC and 1.0ml cavy serum (1:10) were mixed with the splenocyte suspension, and kept at 37°C for 1 hour. After centrifugation, the absorbance of the supernatant was measured at 413nm [16].

Hemolysin in serum assay. Hemolytic assays were performed using a modification of Zhang's method [17]. 5ml 20% SRBC was injected into the mice twice at a seven days interval for immunity. The serum of each mouse was divided equally into two parts. One part was added into equal volumes of mercaptoethanol. Then, the solution was incubated at 37°C for 30 min to destroy IgM, and diluted 500 times. 0.5ml IgG (1:25), 0.5ml cavy serum (1:5) and 1.0ml physiological saline were added into a mixture of 0.5ml of the diluted solution treated with 2-mercaptoethanol and 0.5ml 5%SRBC to determine IgG hemolysin. 0.5ml of another part of serum from the treated mice by 20% SRBC, 0.5ml 5%SRBC, 0.5ml cavy serum (1:5) and 1.5ml physiological saline were mixed to determine IgM serum. All samples for assay of IgG and IgM serum were incubated at 37°C for 1 hour, and shaken once at the middle of time. The samples were then centrifuged at 3000 × g for 10min, were determined. All measurements of the supernatants were made at 540 nm. IgG hemolysin (HCIgG) and IgM hemolysin(HCIgM) were calculated as follows,

$$HC_{IgG} = A_m \times n \quad (2)$$

$$HC_{IgM} = A_b \times n \quad (3)$$

where Am was absorbance of the serum treated by mercaptoethanol, Ab was absorbance of the serum without treatment of mercaptoethanol, n was the diluted times of serum.

UV-Visible spectrum analysis.UV-Visible spectrum was obtained in a Shimazu UV-265 spectrometer.

Statistical analysis. The data were analyzed statistically using SAS8.2. The level of significance was at a P value less than 0.05.

Results

The effect of rapeseed polyphenol on Tumor inhibition. A significant tumor regression was observed at three doses of polyphenol L –0, 50 mg/kg of polyphenol L -I and 50 mg/kg of polyphenol L –IIwhile compared with model control (Figure 1). Tumor inhibition rates of polyphenol L –0 at the doses of 50-200 mg/kg were ranged from 30.23% to 44.19%, and exhibited a dosage effect. It indicated that rapeseed polyphenol could inhibit significantly the tumor although the tumor inhibition rates at polyphenol L –0(three dosage), L -I and L –II were less than that of Cy at the dosage of 20 mg/kg (Table 1). For a dosage of 50 mg/kg, the tumor inhibition rate of polyphenol L -Iwas stronger than these of polyphenol L –0 and L –II.

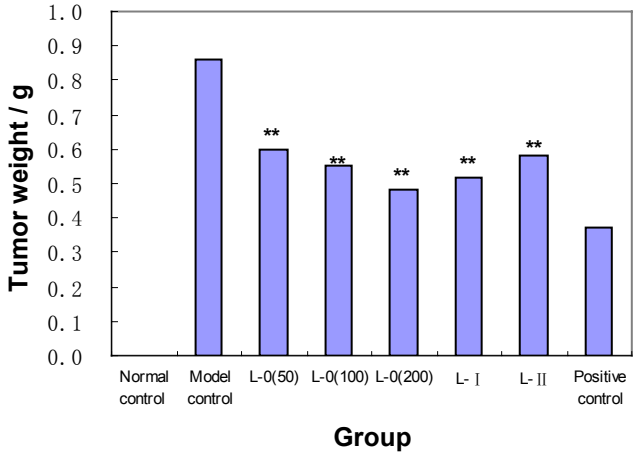

Figure 1. Effect of the rapeseed polyphenol on tumor regression of tumor-bearing mice. Model and positive control and the polyphenol groups were inoculated sarcoma 180, normal control not. Model control received saline intraperitoneally and positive control cyclophosphamide. The rapeseed polyphenol was dissolved in saline and was administered intraperitoneally. The dose volume was 0.2 ml. Data are expressed with means±S.E. of 10 mice, $P < 0.01$ vs. model control.

Table 1 Characteristics of tumor inhibition of rapeseed polyphenol on the mice inoculated Sarcoma 180.
Values are means±S.E. of 10 mice.

Group	Body weight gain (g)	Tumor inhibition rate (%)
Normal control	11.42±2.02	/
Model control	10.76±1.46	/
L-0 (50 mg/kg)	8.65±2.77	30.23
L-0 (100 mg/kg)	7.89±1.27	36.05
L-0 (200 mg/kg)	9.32±1.64	44.19
L-I (50 mg/kg)	8.80±2.90	39.53
L-II (50 mg/kg)	8.92±2.55	32.56
Positive control (Cyclophosphamide)	7.45±1.57	57.00

Rapeseed polyphenol was an anti-nutrition substance in that rapeseed polyphenol can combine with protein to influence the digestion and absorption of protein [18]. However, there were no significant difference at the index of body weight gain between rapeseed polyphenol group (polyphenol L -0(a), L -I (b), and L –II (c)) and normal control group (Table 1). Possibly because rapeseed polyphenol was injected into the axilla of mice rather than entrance through alimentary canal, rapeseed polyphenol had no chance to contact the nutrition to influence the digestion and absorption of nutrition.

Pathology observation of tumor tissue. The tumor inhibition effect of rapeseed polyphenol was also demonstrated by the micrograph of tumor tissue through HE coloration. Under the high power microscope, karyon showed black color, dyed tumor cells exhibited deeply red color and irregular profile, and some of tumor cells displayed carykinesis phase (Figure 2A), which were the characteristics of tumor during a flourishing growth. The region in the upside of Figure 2B showed lightly red color, there was only a few of purple dots and no full karyon of tumor after the administration of Cyclophosphamide, the purple dots were the residues of necrotic tumor cells. And under the high power microscope, the region of necrotic tumor cell, being lightly red color, exhibited in form of strip and piece (Figure 2E-2I). The dead tumor cells, not owing to necrosis, also showed lightly red color region in control group (Figure 2C), but the area of the region with lightly red color for control group (Fig.4c) was smaller than that of every rapeseed polyphenol group (Figure 2D-2H), it indicated that rapeseed polyphenol inhibited significantly the tumor cell growth, although the inhibition effect of rapeseed polyphenol was lower than that of Cyclophosphamide(Figure 2D). This result of images was identical with that of tumor weight. Simultaneously, the area of lightly red region exhibited a significant dosage effect at three dosages of rapeseed polyphenol L-0.

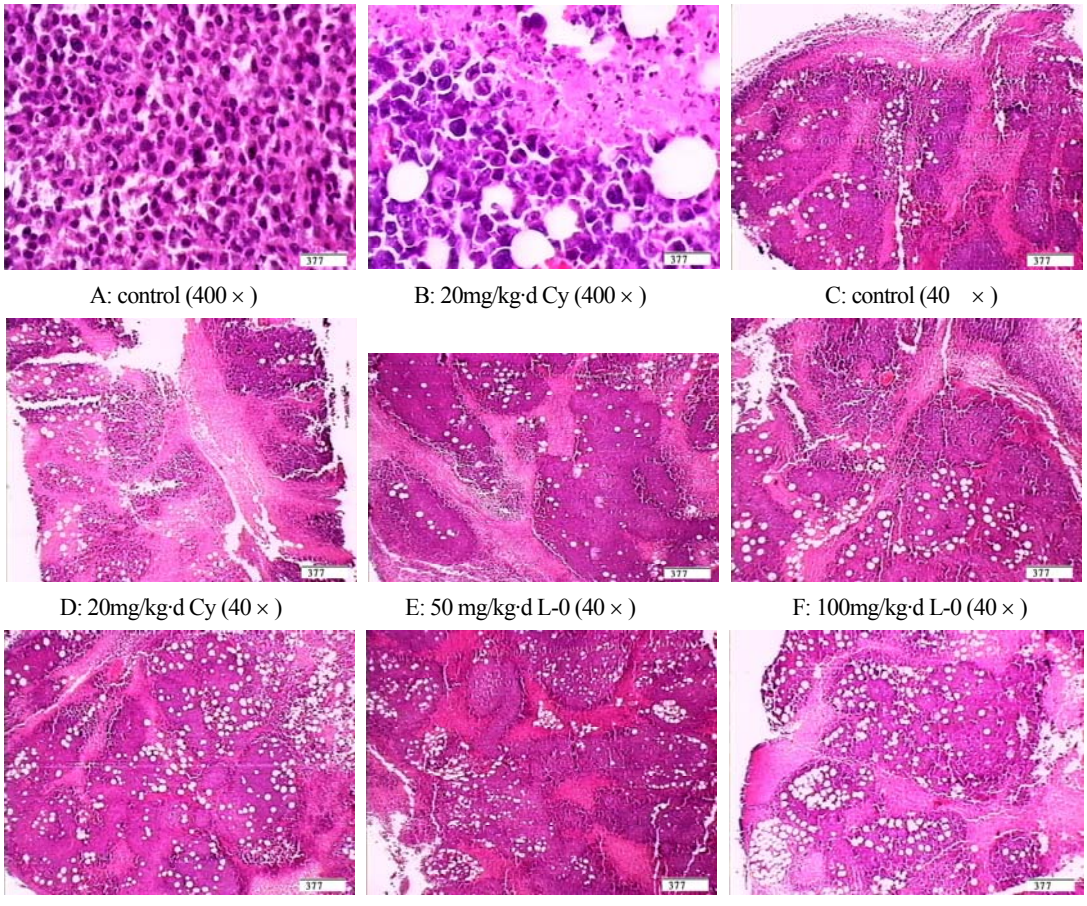

Figure 2. Micrograph of tumor tissues of various group mice treated with substance at various amplified times. a-i represented control group(no treatment, 400 ×), Cyclophosphamide (20 mg/kg, 400 ×), control group (40 ×), Cyclophosphamide (20 mg/kg, 40 ×), polyphenol L -0 (50 mg/kg, 40 ×), polyphenol L -0 (100 mg/kg, 40 ×), polyphenol L -0 (200 mg/kg, 40×), polyphenol L -I (50 mg/kg, 40 ×), and polyphenol L-II (50 mg/kg, 40 ×), respectively.

Thymus and spleen weight. Thymus and spleen is immunity organ. Their weights reflect non-specific immunity function. At the dosage of 50, 100, and 200 mg/kg polyphenol L -0, a significant increase ($P<0.05$) in relative organ weight of thymus and spleen was observed (Table 2).

Table 2. Effect of the rapeseed polyphenol on relative spleen and thymus weight of tumor-bearing mice. Relative thymus weight was measured in the ratio of the thymus weight (mg) to body weight (g). Relative spleen weight was measured in the ratio of the spleen weight (mg) to body weight (g). Values are means±S.E. of 10 mice.

Group	Relative thymus index (mg/g)	Relative spleen index (mg/g)
Normal control	3.32±0.43	6.63±1.19
Model control	2.99±0.52	8.64±1.31
L-0(50 mg/kg)	3.21±0.90	9.21±1.83
L-0(100 mg/kg)	3.27±0.52	9.01±1.50
L-0(200 mg/kg)	3.34±0.98	9.13±1.46
L-I(50 mg/kg)	3.19±1.00	9.93±1.53
L-II(50 mg/kg)	3.13±0.96	9.17±1.72
Positive control (Cyclophosphamide)	1.84±0.39	5.44±0.84

Phagocytosis of macrophage. Phagocytosis ability of macrophage increased significantly for the tumor-bearing mice treated with rapeseed polyphenol L-0, IorIIas compared with model group (Figure 3).

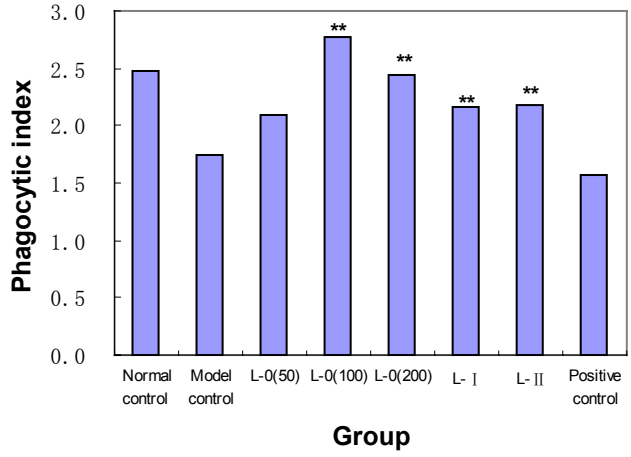

Figure 3. Effect of rapeseed polyphenol on phagocytosis of macrophage. The phagocytic index was measured by counting the number of phagocytosed CRBC per 100 macrophage cells. Values are means±S.E. of eight mice; **P＜0.01 vs. model control.

Delayed-type hypersensitivity reaction. Administration of five group of rapeseed polyphenol, a significant increase of weight difference is found while comparing to model control group(Figure 4).

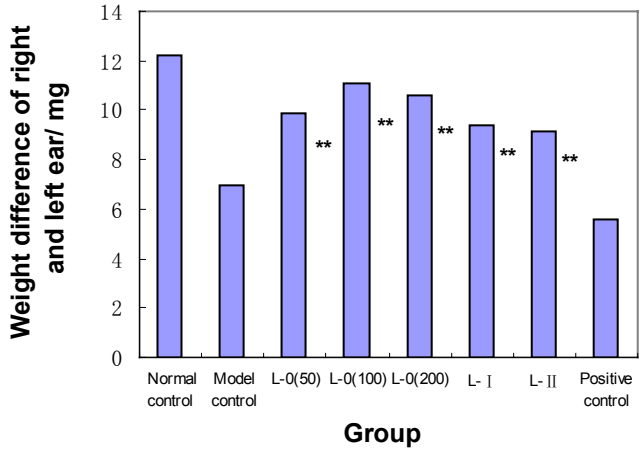

Figure 4. Effect of rapeseed polyphenol on cell immunity (evaluated by DTH to DNFB). Mice were sensitized to dinitrofluorobenzene (DNFB). The antigen challenge was evaluated by measuring weight difference of right and left ear with an analytical balance. Values are means±S.E. of 10 mice; **P＜0.01 vs. model control.

Splenocyte antibody formation. To confirm the effect of the rapeseed polyphenol on the cellular immune response, the antibody formation of splenocytes is evaluated from mice. The formation of splenocyte antibody shows the ability of B cell to secrete antibody and the whole immune function of boby fluid [19]. The results indicated that the antibody index of polyphenol groups increased significantly comparing to model group, whereas for polyphenol L-0, their responses were almost restored to normal level (Figure 5).

Hemolysin content in serum. Hemolysin content in serum reflects the special immune function of body fluid. From table 3, it is shown that only polyphenol L-0 at high concentration enhanced significantly the content of hemolysin. It implied that rapeseed polyphenol did not increase the immune function through hemolysin effect. However, cyclophosphamide signidicantly reduced the immune function through decreasing hemolysin content.

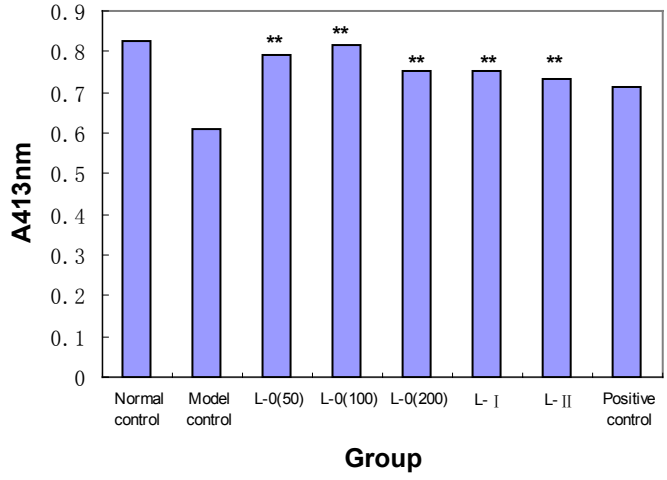

Figure 5. Effect of rapeseed polyphenol on the formation of splenocyte antibody. The concentration of Splenocyte antibody was expressed as the absorption at 413 nm. Values are means±S.E. of 10 mice; **P＜0.01 vs. model control.

Table 3 **Effect of rapeseed polyphenol on content of serum hemolysin in Sarcoma180 bearing mice.** ***Values are means±S.E. of 10 mice. P＜0.05 vs. model control..***

Treatment	HC_{LgG}	HC_{LgM}	$HC_{LgG}+HC_{LgM}$
Normal control	229.6±33.7	525.7±79.2	755.3±118.7
Model control	198.4±21.0	414.3±63.0	612.7±79.8
L-0(50 mg/kg)	213.3±21.9	425.5±53.6	638.8±72.4
L-0(100 mg/kg)	214.6±18.4	434.7±50.9	649.3±65.9
L-0(200 mg/kg)	229.1±22.3	478.3±53.6	707.4±73.6
L-I(50 mg/kg)	214.1±23.4	426.4±45.9	640.5±68.1
L-II(50 mg/kg)	213.1±18.9	426.4±46.2	639.5±63.7
Positive control (Cyclophosphamide)	178.7±18.7	215.2±57.6	393.9±73.1

Lactic dehydrogenase activity. In this study, lactic dehydrogenase activity in the serum of sarcoma 180 mice decreased significantly after administration of rapeseed polyphenols (polyphenol L-0, L-I, L-II). Rapeseed polyphenol inhibited lactic dehydrogenase activity(Figure 6).

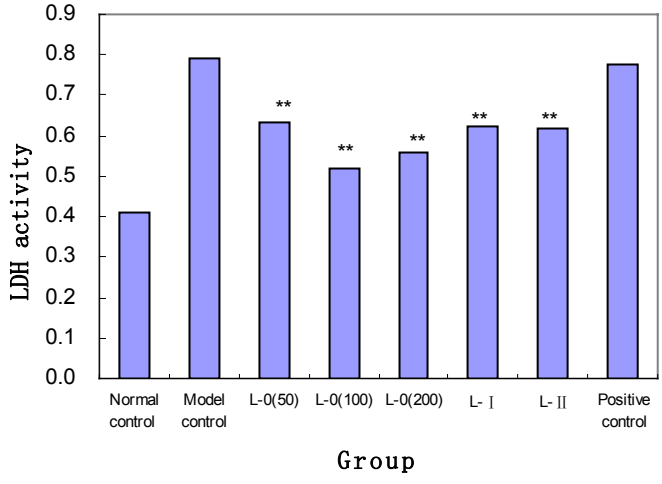

Figure 6. Effect of rapeseed polyphenol on the activity of serum lactic dehydrogenase in S180 bearing mice. *Values are means±S.E. of 10 mice; ** P＜0.01 vs. model control.*

Catalase activity. The CAT activity of Erythrocyte was assessed by H_2O_2 consuming. The rapeseed polyphenol showed a significant effect on CAT activity (Figure 7).

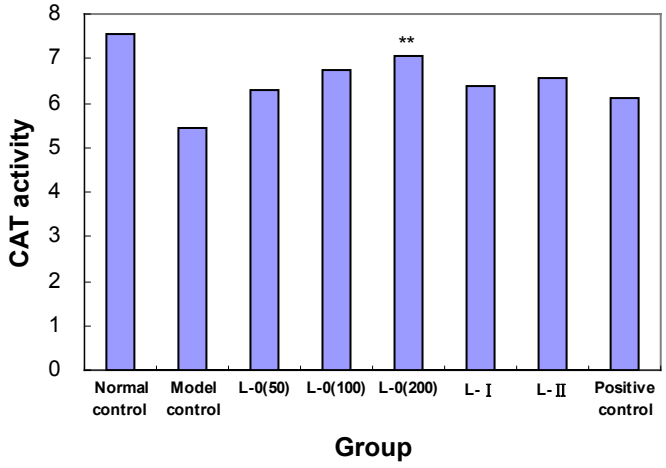

Figure 7. Effect of rapeseed polyphenol on the activity of catalase in erythrocyte of S180 bearing mice. *Values are means±S.E. of 10 mice; **P <0.01 vs. model control.*

Discussion

A tumor-bearing animal model is made to research on the antitumor and immunomodulating activities according to pharmacology. A significant tumor regression of the rapeseed polyphenol L-0 (50, 100, and 200 mg/kg), L-I(50mg/kg), and L-II(50mg/kg) group mice was observed comparing with model control group (Figure 1). The tumor inhibition effect of rapeseed polyphenol was also demonstrated by the micrograph of tumor tissue through HE coloration. It indicated that rapeseed polyphenol inhibited significantly the tumor cell growth, although the inhibition effect of rapeseed polyphenol was lower than that of cyclophosphamide (Figure 2D). This result of images was identical with that of tumor weight. Simultaneously, the area of lightly red region exhibited a significant dosage effect for rapeseed polyphenol L-0.

A significant increase of relative thymus and spleen weight in the polyphenol groups' mice was observed comparing with model control group (Table 1). The relative spleen and thymus weight were important index for nonspecific immunity. Immunopotentiator could increase spleen and thymus weight. Immunosuppressive agent could induce weight decrease of spleen and thymus or decline for immune function. The polyphenol could restore the immunity of mice, which was of the inhibition of tumor retain unclear.

It is confirmed that the polyphenol could augment phagocytosis of macrophage of tumorbearing mice (Figure 3), and Delayed-type hypersensitivity reaction enhanced significantly through administration of the polyphenol L-0, I, or II (Figure 4). The formation of splenocyte antibody shows the ability of B cell to secrete antibody and the whole immune function of boby fluid (19).Our research indicated that the antibody index of tumor-bearing mice treated with rapeseed polyphenol also increased significantly. For polyphenol L-0, the index was almost restored to normal level (Figure 5). Moreover, Hemolysin content in serum reflects the special immune function of body fluid. Table 3 showed that only polyphenol L-0 at high concentration enhanced significantly the content of hemolysin. It implied that rapeseed polyphenol did not increase the immune function through hemolysin effect. However, cyclophosphamide significantly reduced the immune function through decreasing hemolysin content.

Due to relative thymus and spleen weight, and phagocytosis index of macrophage reflect non-specific immune function of body, delayed-type hypersensitivity reaction reflects the cellular specific immune function, and hemolysin in serum reflect reflects the immune function of body fluid. All of these indexes show the whole immune function of body. The results of these indexes demonstrated that rapeseed polyphenols could enhance the immune function.

Lactic dehydrogenase is a key glycolytic enzyme. The energy for tumor cell's growth depends mainly on glycolysis since tumor cells will gangrene without nutrition [20]. Therefore, lactic dehydrogenase activity usually enhanced when tumor cells appeared. In our research, lactic dehydrogenase activity in the serum of sarcoma 180 mice decreased significantly after administration of rapeseed polyphenols (polyphenol L-0, L-I, or L-II). Rapeseed polyphenol inhibited lactic dehydrogenase activity, which availed to inhibit sarcoma 180 growth, but rapeseed polyphenol inhibited sarcoma 180 growth not only through the inhibition of lactic dehydrogenase activity because the lactic dehydrogenase activity did not exhibit a dosage effect for polyphenol L-0. As above experiments, cyclophosphamide inhibited efficiently tumor growth, but the lactic dehydrogenase activity did not change significantly after the administration of cyclophosphamide. It implied that the inhibition of sarcoma 180 growth treated with cyclophosphamide did not depend on mainly lactic dehydrogenase activity effect.

Catalase, an antioxidase, can catalyze hydrogen peroxide to be degraded as water so that the body is avoided to be oxidized. The rapeseed polyphenol L-0, Ior II could increase the catalase activity of in erythrocyte of sarcoma

180 bearing mice.(Figure 7). It demonstrated the antitumor activity of rapeseed polyphenol attributed to antioxide ability of macrophage partly.

Acknowledgement

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References

[1] Nergarda, C. S.; Diallo, D.; Inngjerdingen, K.; Michaelsen, T. E.; Matsumoto, T.; Kiyohara, H.; Yamada, H.; Paulsen, B. S. Medicinal use of Cochlospermum tinctorium in Mali Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology*, **2005**,96: 255–269

[2] Lambert, J. D.; Yang, C. S. Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. Mutation Research, **2003**, 523–524:201–208

[3] Zdunczyk, Z.; Frejnagel, S.; Wroblewska, M.; Juskiewicz, J.; Oszmianski, J.; Estrella, I. Biological activity of polyphenol extracts from different plant sources. *Food Research International*, **2002**, 35: 183-186

[4] Sakihama, Y.; Cohen, M. F.; Grace, S. C.; Yamasaki, H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals implants. *Toxicology*, **2002**, 177: 67–80

[5] Yan, F.-W.; Wu, M.-C.; Jiang, H.; Wu, J.-Q. Compositive extraction of rapeseed cake. *Transaction of Chinese Society of Agricultural Engineering*, **2004**, *20*(2): 209-212

[6] Udaya, N. W.; Ryszard, A.; Fereidoon S. Partial characterization of natural antioxidants in canola meal. *Food research international*, **1996**, *28*(6):525-530

[7] Yan, F.-W.; Luo Z.-Y.;Xue Z.-H.;Wu, M.-C. Antioxidation of polyphenol from rapeseed. *China Oils and Fats*, **2005**, *30*(7): 54-57

[8]Yan, F.-W.; Luo Z.-Y.; Xue, Z.-H.; Wu, M.-C. Antioxidation effect of polyphenol-1 from rapeseed and the antioxidation mechanism. *Journal of the Chinese Cereals and Oils Association*, **2005**,*20*(5):115-119

[9] Li, W.-D.; Ren, L.-S.; Lin, Z.-B. Antitumor activity and immunopharmacological effects of Linglong liquidum extractum (LLE). *Chin J New Drug*, **2002**; 11: 928– 31.

[10] Mihich, E.; Ehrke, M. J. Anticancer drugs plus cytokines: immunodulation based therapies of mouse tumors. *International Journal of Immunopharmacology*, **2000**, 22:1077-1081

[11] Pang, Z.-J.; Zhou M.; Cheng Y. In *Method of ROS research*, People's Medical Publishing House, Beijing, China, 2000, pp 61– 150.

[12] Li, X.-D.; Rong J.-H.; Wu, M.-C.; Zeng X.-B. Anti-tumor Effect of Polysaccharide from Grifola Frondosa and Its influence on Immune Function. *Journal of Chinese Medicinal Materials*, **2003**, *26*(1):31-32

[13] Zeng, X.-B.; Wu, M.-C.; Li, X.-D. Effect of rapeseed peptides on tumor growth and immune function in mice. *Acta Nutrimenta Sinica*, **2002**, *24*(4): 405-407

[14] Zhang, J.-T.. In *Modern experimental methods in pharmacology*. Beijing Medical University and Peking Union Medical College Press; Beijing, China, 1997.

[15] Fan, M.-Z.; Li, C.-R.; Li, Z.-Z.; Lu, Z.-M.; Shi, G.-Y.; Chen, X.-.X. Evolution on immunity function of strong cordyceps mecylium capsules. *Microbiology*, **2002**, 7:19– 22.

[16] Xu, S. In *Experimental methods for Pharmacology*, People's Medical Publishing House, Beijing, China, 1982

[17] Zhang, C.-J.; Zhou, G.Y.; Li, H.; Guo, C.H.; Li, L. Associating antimutor effect of Grape polyphenol and amycin in mice with liver cancer(H22). *Journal of Shangdong University (Health Sciences)*, **2002**, *40*(5): 474-475

[18] Augustin, S.; Christine, M.; Claudine, M.; Christian,. R. Absorption and metabolism of polyphenols in the gut and impacton health. *Biomed Pharmacother*, **2002**, 56: 276–282

[19] Yan, M.-X.; Chen, Z.-Y.; Xian, B.-K.;Huang, X.-M. Effect of Flammulina Velutipes polysaccharide on serum hemolysin and cell of antibody formation in mice. *Information on Traditional Chinese Medicine*, **2003**, *20*(5): 56-57

[20] Jin, L.-Q.; Lu, J.-X.; Yang, J.Z.; Li, D.; Li, A.-L. Experimental study on the regulative effect of total polysaccharides of paecilomyces cicadidae on immunologic function in old rats. *Chinese Journal of Gerontology*, **2005**, *25*(1): 82-84

Impact of conventional breeding on the whole seed proteome of *Brassica napus* L. using quantitative differential 2D electrophoresis and shotgun proteomics

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Abstract

In this study, four near-isogenic *B.napus* varieties with absence of erucic acid and decreasing amounts glucosinolates were used to characterise the proteins affected during the breeding process. Two strategies were investigated, 1/ by quantitative differential 2D electrophoresis and 2/ by shotgun proteomic approach involving isobaric tagging of peptides.

1) Using 2D electrophoresis analysis, 72 spots were found to be differentially expressed between the studied lines. Forty were identified by mass spectrometry that are involved mainly into carbohydrate pathway or detoxification/ defence. A validation of the differential expression of two of these proteins was performed using quantitative PCR.

2) Despite the high performance of 2D electrophoresis, it was difficult to quantify the storage proteins. Cruciferins were scattered into at least 20 distinct spots and napins were hardly detected due to their low molecular mass and the basicity of some isoforms. In this context, we used an alternative shotgun proteomic approach which is based on the enzymatic hydrolysis of the entire proteome, followed by a multi-dimensional chromatographic separation of the resulting peptides. In order to compare the expression level of proteins between the four lines, peptides were labeled with iTRAQs reagents prior to tandem mass spectrometry. Results show that the ratio between cruciferins and napins in mature seeds correlates to the amount of glucosinolates.

Key words: Oilseed rape, *Brassica napus*, 2D electrophoresis, breeding, napin, cruciferin, shotgun proteomic, iTRAQ

Introduction

Rapeseed was subjected to intensive selective breeding to reach what is called canola-quality rapeseed (double-low "00", "zero erucic acid, zero glucosinolates") with the reduction in two toxic compounds : the erucic acid in the edible oil was eliminated, and the glucosinolate level was decreased in the meal for feedstock uses. Brassica napus seeds contain a series of glucosinolates derived from various amino acids. These secondary metabolites are involved in defence against predators or parasites through their degradation products (Bone & Rossiter, 1996) which are also toxic in nutrition. After rapeseed industrial transformation, glucosinolates and their degradation products still remain in meal. The improvements made through breeding research programs paid little attention to the seed protein composition. However previous studies showed that the balance in the major storage proteins (napins (2S) and cruciferins (12S)) has been modified into the 00 varieties (Raab et al., 1992; Malabat et al., 2003).

Previous studies comparing varieties by 2D electrophoresis pointed out a large number of protein variations, new isoforms, displacement of protein probably related to different post translational modifications and a very large number of quantitative differences. In the case of Arabidopsis, a proteomic comparison of eight ecotypes revealed that they displayed qualitative as well as quantitative differences. They share only 25% of their total spots and 10 % of them were specific for one ecotype (Chevalier et al., 2004). The number of common spots increased considerably when the comparison is achieved between varieties of the same species. Two *B. oleracea* were shown to share around 77 % of their leave proteome and 78 % of their stem proteome (calculated from Albertin's data, 2005).

In our study, we decided to explore the impact of the breeding process (reduction of erucic acid and glucosinolate contents) on the seed proteome by using two strategies : 1) quantitative differential 2D electrophoresis and 2) shotgun proteomic approach involving isobaric tagging of peptides. Despite the well known efficiency of 2D electrophoresis, the second strategy was developed in order to get information on the 2S storage protein (napin) which, because of their high basicity added to their small molecular weight, are not detected in 2D gels.

Material and Methods

Plant material and growth conditions. Four winter-type *Brassica napus* varieties, namely Gaspard (++), JetNeuf (0+), Darmor (00) and Darmor-*bzh* (00) that belong to a near-isogenic family, provided by APBV INRA Research Centre in Rennes, France, were used in this study (Renard et al., 2001). Plants were grown under controlled and reproducible conditions (16-hours photoperiod; 18 to 20°C night/20 to 22°C day temperature). Dry mature seeds were harvested at 60 days after pollination.

2D electrophoresis. Dry mature *Brassica napus* seeds were ground in a buffer (8M Urea, 2% Chaps, 2M Thiourea,

18mM DTT, 2% ASB C80). After 2 hours agitation at room temperature, the extracts were centrifuged at 13,000g for 30 min and the protein fraction was recovered in the supernatant.

Isoelectrofocusing was performed on strips pH 3-10 loaded with 200 or 500 µg of proteins. The proteins were separated in a second dimension on 10% and 15% polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue G250 (Sigma-Aldrich) according to Consoli & Damerval, 2001.The images obtained from the four varieties were analysed using Image Master 2D Platinium software (GE Healthcare). The statistical analysis were performed using Statgraphics. Protein spots were picked up manually and in gel digested by trypsin prior to mass spectrometry. The resulting peptide mixture was acidified by the addition of 1 µL of an aqueous solution of formic acid (1%, vol.).

Sample preparation and labeling for shotgun analysis. One hundred micrograms of protein from each sample were reduced, alkylated, digested with trypsin and labeled with the isobaric reagents according to the protocol given in the iTRAQ™ reagent kit (Applied Biosystems). Labeled samples were pooled and subjected to strong cation exchange (PolyLC Polysulfoethyl A column (4.6 mm x 100 mm) fractionation with salt gradient (Agilent Technologies, Inc, Palo Alto, CA, USA). Collected fractions were dried using a vacuum centrifuge. Samples were labeled as follows: 114 tag – $Darmor_{bzh}$; 115 tag – Darmor; 116 tag – Jetneuf; 117 tag – Gaspard

Protein identification by mass spectrometry. The digested proteins and the labelled peptides mixture were analysed on a nanoscale capillary liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Switchos-Ultimate capillary LC system (LC Packings/Dionex, Amsterdam, the Netherlands), coupled with a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF Global, Micromass/Waters, Manchester, UK). Mass data acquisitions were processed with Masslynx software (Micromass/Waters) using the so-called "data dependent acquisition" mode. Protein identification was achieved by searching mass data in the UniProt/Swiss-Prot and UniProt/TrEMBL databanks (10-01-2006), or in the TIGR Gene Indices databank (Brassica napus: release: 29-09-2004).

Gene expression quantification using real-time quantitative PCR. Total RNA was extracted with the SV Total RNA Isolation System (Promega, Madison, USA) according to the instructions of the manufacturer. For reverse transcription (RT)–PCR studies, 2.5 µg of DNA-free RNA extract was converted into first-strand cDNA by using the SuperScriptII preamplification system for first-strand synthesis kit (Invitrogen) and $oligo(dT)_{12–18}$. Amplification of cDNA was conducted in optical 96-well plates with an ABI PRISM® 7700 cycler (Perkin-Elmer Applied Biosystems, Foster City, CA) using SYBR® Green to monitor dsDNA synthesis. Reactions were performed with 12.5 µL 2x SYBR Green Master Mix reagent (Applied Biosystems), 12.5 ng of cDNA and 900 nM of each primers in a final volume of 25 µL, under the following thermal conditions : 50°C for 2 min; 95°C for 10 min; 40 cycles of 95°C for 15 sec and 60°C for 1 min. At the end of the reaction, a dissociation curve (95°C for 15 sec; 60°C for 20 sec; a 20 min-ramp to 95°C; 95°C for 15 sec) was run to verify amplicon specificity. Data were analysed using the SDS 1.7 software (Perkin-Elmer Applied Biosystems). To determine the level of specific transcripts present in a sample, we performed relative quantification through the method of the standard curve (according to ABI7700 user guide). Gene specific primers were designed with Primer Express 1.5 software (Perkin-Elmer Applied Biosystems) using the basic parameters (primer size 18-25 bases; 20-80% GC; primer Tm 58-62°C; amplicon length 50-150 bp; amplicon Tm > 76°C). Sequences are as follows:

Q-BnEF1-1-UP 5′-CGGTGTCATCAAGAGCGTTG-3′; Q-BnEF1-1-RP 5′-GGACTTTGATTCATCTGCGGAT-3′; b-glucosidase-UP 5′-CCGGGCCTAAAACAGAAATCAT-3′; b-glucosidase-RP 5′-TGAGAAGAACTCCAACACAAACTCA-3′; Glutathione S transferase-UP 5′-CCCTGGGTATGAGAAGTTTGGT-3′; Glutathione S transferase -RP 5′-CCTCTTAGCCCATGCAATCAG-3′

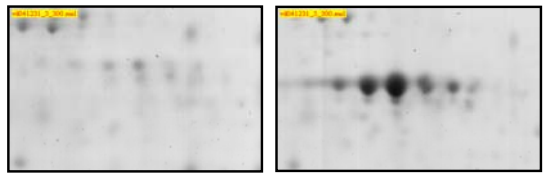
Results and discussion

2D electrophoresis reveals slight differences in protein expression within the near isogenic line

The seed proteomes of the four varieties were compared using 200 µg protein loading and 15% acrylamide gels. A mean number of spots of 180 was obtained in these conditions. Five very intense spots were present on these gels; using western blot analysis they were identified as cruciferin polypeptides. This identification has been confirmed and refined by mass spectrometry. The cruciferins polypeptides were scattered into about 20 spots as revealed by Western blot analysis. The sum of the volumes of these spots accounted for about 30 % of the total spots volume on the gels obtained for all the varieties. Only two of them identified as cruciferin beta polypeptides were differentially expressed between the varieties.

In order to get deeper into the seed proteome, the varieties were compared using 500 µg of protein loading and 10% acrylamide gels. These conditions permitted to increase the number of spots. A slightly but significantly different number of spots was detected between Gaspard, JetNeuf, Darmor and Darmor-*bzh* with 495 ± 40, 456 ± 29, 562 ± 8 and 604 ± 32 spots, respectively. A multiple range test at 0.01 confidence level revealed a total of 69 differences between these varieties.

Gaspard (++) and Jetneuf (0+) differ by their acid erucic content, and present 55 different spots and most of them were over expressed in Jetneuf. Among these, 32 proteins were identified and are mainly involved in two functional classes : carbohydrate pathway and detoxification/ defence system.



Gaspard Jetneuf

Spots on Darmor with their identification numbers

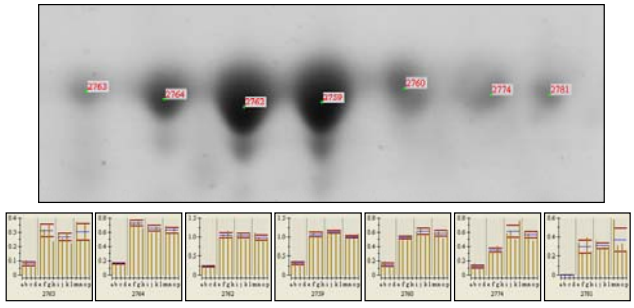


Fig 1 : Zoom on seven spots of TC358 homologue to beta glucosidase Q42618 in a 2D gel electrophoresis.
Expression pattern of each spot across the four varieties : Gaspard, Jetneuf, Darmor, Darmor bzh.

Beta-glucosidase class 1 (TC 358 in the TIGR gene indices) is an example of proteins expressed differentially between the varieties (Figure 1). Beta glucosidase is implicated in the polysaccharide catabolism and such a large variation in its expression between Gaspard and its progeny was not expected.

Therefore we quantified its mRNA expression by RT PCR within the four varieties during the last developmental stages (Figure 2).

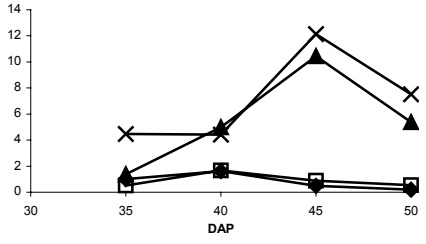


Fig 2 : Relative expression profiles of beta glucosidase (Q42618) measured in Gaspard (rhombus), Jetneuf (squares), Darmor (triangles) and Darmor bzh (crosses)

The expression patterns of beta glucosidase in the mature seeds were quite similar across the seven spots, this protein is clearly under expressed in Gaspard. The comparison with mRNA expression patterns shows that the mRNA level is very low for Gaspard and higher for Darmor and Darmor$_{bzh}$. However the mRNA expression pattern in JetNeuf is comparable to that in Gaspard. It seems that in this case, the transcripts are present but not their products.

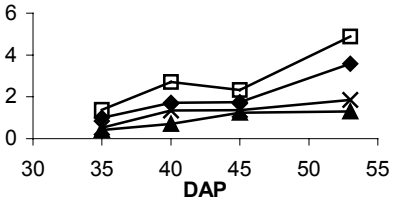


Fig 3 : Expression profiles of glutathione S transferase (Q9ZRW8) measured in Gaspard (rhombus), Jetneuf (squares), Darmor (triangles) and Darmor bzh (crosses)

The differential expression of beta glucosidase within the near isogenic line was confirmed. The relation with the breeding process is quite unclear, although a potential involvement of these enzymes in stress response and plant defence has been recently suggested (Stotz et al., 2000; Matsushima et al., 2003),.

Among the proteins involved in the defence/detoxification system, we focused here on a glutathione S transferase (GST, Q9ZRW8) over expressed in double null varieties compared to their parents. In this case a good correlation was found with the corresponding mRNA patterns (Figure 3). Many functions have been described for GSTs : conjugation between G-SH and xenobiotics, defence against oxidative stress and involvement in plant cell stress signaling. Taking into account these functions

and especially those of detoxification, it cannot be excluded that the less amount of GST in double null lines could be a consequence of the breeding process.

As expected, the use of varieties belonging to a near-isogenic family lead to a reduction of the proteome variability. Lowering the background we found 39 differentially expressed proteins however in most of cases it was difficult to rely their expression and their functions to the breeding process. In the case of storage proteins, our results didn't revealed any impact on the 12S globulin family.

Comparison by shotgun proteomic method associated to iTRAQ labelling

Using 2D electrophoresis, we were able to quantify and compare only one of the two major storage protein families in the seed, the 12S globulin. However some papers reported a variation of the ratio between 12S and 2S related to the breeding for glucosinolates. Therefore as napins (2S) were hardly detected due to their low molecular mass and the basicity of some isoforms, we set up an alternative shotgun proteomic method. The entire seed proteome of each variety is first hydrolysed, the resulting peptides are then labeled with one of the four isobaric tag and the derivatized digests are combined together. This mixture is then separated by a multi-dimensional chromatography prior mass spectrometry analysis.

Table 1. Seed storage proteins relative quantification (± 0.2) between the four varieties.

	G/J (++/0+)	G/D (++/00)	J/D (0+/00)	D/Dn (00/00)
	117/116	117/115	116/114	115/114
12S family	0.84	0.81	0.92	1.09
2S family	1.04	1.53	1.66	1.01

This strategy resulted in 4000 spectra (three repetitions) from which 457 peptides were identified and quantified. Among these, 66 originated from storage proteins and permitted the identification of 13 different storage proteins. In the case of the 2S family, 7 different napins were identified, each by at least three specific peptides. Their relative quantification between the four varieties revealed that most of these proteins were less expressed in the low glucosinolates varieties. Another analysis of our results was also performed by grouping peptides of the same family and used all of them to quantify the family. The results obtained for 2S and 12S families are reported in Table 1. The relative quantification across the four lines revealed a relative stability of the expression of the 12S family given the maximum standard deviation of 20% (Ross et al., 2004). This was also the case when looking at the isoforms individually. On the contrary, the expression of the 2S decreased in the double null varieties lowered in their glucosinolates content.

Conclusion

Numerous proteins differentially expressed have been identified and quantified. The two strategies developed here are very complementary for the type of proteins detected. Some proteins were identified by both methods; the measurement of their differential expression give very similar results and therefore reinforce our findings. The expression of napin decreased in varieties selected for their lower amount in glucosinolates while at the same time the expression of globulins remained stable. These results corroborate those on the increase of the 12S/2S ratio in double null varieties compared to their parents.

References

Renard, M.; Delourme, R.; Barret, P.; Brunel, D.; Froger, N.; Tanguy, X.(2001) Mutant gene of the gras family and plants with reduced development containing said mutant gene. WO 0109356, 2001.

Raab, B.; Leman, H.; Schwenke, K. D.; Kozlowska, H. Comparative study of the protein patterns of some rapeseed (Brassica napus L.) varieties by means of polyacrylamide gel electrophoresis and high-performance liquid chromatography. Nahrung 1992, 36, (3), 239-247.

Malabat, C.; Atterby, H.; Chaudhry, Q.; Renard, M.; Guéguen, J. Genetic variability of rapeseed protein composition. In 11 th International Rapeseed Congress. Towards Enhanced Value pf Cruciferous Oilseed Crops by Optimal Production and use of the High Quality Seed Components, Copenhagen, DNK, 2003; Sorensen, H.; Sorensen, J. C.; Sorensen, S.; Bellostas Muguerza, N.; Bjergegaard, C., Eds. pp 205-208.

Albertin, W.; Brabant, P.; Catrice, O.; Eber, F.; Jenczewski, E.; Chevre, A. M.; Thiellement, H. Autopolyploidy in cabbage (Brassica oleracea L.) does not alter significantly the proteomes of green tissues. Proteomics 2005, 5, (8), 2131-9.

Chevalier, F.; Martin, O.; Rofidal, V.; Devauchelle, A. D.; Barteau, S.; Sommerer, N.; Rossignol, M. Proteomic investigation of natural variation between Arabidopsis ecotypes. Proteomics 2004, 4, (5), 1372-81.

Stotz, H. U.; Pittendrigh, B. R.; Kroymann, J.; Weniger, K.; Fritsche, J.; Bauke, A.; Mitchell-Olds, T. Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of Arabidopsis against Egyptian cotton worm but not diamondback moth. Plant Physiol 2000, 124, (3), 1007-18.

Matsushima, R.; Kondo, M.; Nishimura, M.; Hara-Nishimura, I. A novel ER-derived compartment, the ER body, selectively accumulates a beta-glucosidase with an ER-retention signal in Arabidopsis. Plant J 2003, 33, (3), 493-502

Ross, P.L., Huang, Y.N., Marchese, J.N., Williamson, B., Parker, K., Hattan, S., Khainovski, N., Pillai, S., Dey, S., Daniels, S., Purkayastha, S., Juhasz, P., Martin, S., Bartlet-Jones, M., He F., Jacobson, A., Pappin, D.J. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents.Mol Cell Proteomics. 2004 Dec;3(12):1154-69

A new NIRS method for high throughput analysis of oleic, linoleic and linolenic acid content of single seeds in oilseed rape

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Abstract

A high-throughput Near-Infrared Reflectance Spectroscopy (NIRS) method using an automated sample presentation unit for single seeds of oilseed rape and a new kind of spectrometer equipped with a photodiode array detector was developed. First analyses have been accomplished with a throughput of about 500 seeds per hour. Seeds from segregating F_2 populations of different origin were analysed by NIRS and gas liquid chromatography (GLC). Calibration equations were developed and validated. The coefficients of determination in cross validation were 0.81 for oleic acid, 0.76 for linoleic acid and 0.57 for linolenic acid. The ratio standard deviation/standard error in cross validation (SD/SECV) ranged from 2.4 for oleic acid over 2.1 for linoleic acid to 1.7 for linolenic acid. Using four different external validation sets, the coefficients of determination in external validation varied between 0.44 and 0.88 for oleic acid, between 0.31 and 0.72 for linoleic acid and between 0.11 to 0.33 for linolenic acid. The results show that the new high-throughput method can be applied to predict the oleic and linoleic acid content of single rapeseeds. However, the calibration seed sample set need to be extended by more seeds derived from different crosses and environments before robust results can be expected in routine analysis.

Key words: fatty acids, quality, single-seed NIRS, Near-Infrared Reflectance Spectroscopy, HOLL, high oleic, low linolenic, photodiode array detector

Introduction

Rapeseed with a high content of oleic acid (18:1) and with a low content of linoleic (18:2) and linolenic acid (18:3) has a large potential for applications in the food and oleochemical industry (Möllers 2004). The aim of the present project is to develop a Near-Infrared Reflectance Spectroscopy (NIRS) method for high throughput analysis of oleic and linolenic acid content of single seeds in oilseed rape. Since the fatty acid composition of the seed oil is mainly determined by the genotype of the embryo, such a method could be useful for the non-destructive analysis of segregating F2-seed populations. Fatty acid composition of single rapeseeds has been successfully determined earlier by applying single seed adapters to standard NIRS equipment (Sato et al. 1998, Velasco et al. 1999).

Material and Methods

Technology: Two innovations allow for a non-destructive high throughput determination of fatty acid composition in single seeds of oilseed rape: an automated sample presentation unit (PPM unum, constructed by Ingenieurbüro Steps, Jena, and VDLUFA, Kassel) and a new kind of spectrometer (ZEISS MCS 611). A schematic drawing of the automated sample presentation unit is shown in Figure 1. The tubes of a spindle move through a holding tank containing seeds of oilseed rape. As the tubes are connected to a vacuum pump, a single seed is aspirated by the tube (1) and transported to a set of three optic fibres (2). The outer two fibres transmit white light of a halogen lamp to the sample seed, while the inner fibre transmits the reflected light to a spectrometer. The spectrometer is equipped with a novel photodiode array detector that is able to record the full spectrum (1340 –2000 nm) at once within milliseconds. Finally, the single seed is stripped off into a downspout (3) and arrives at a free position on a microtiter tray, which then moves automatically to the next free position.

Calibration development: Four breeding companies involved in this project provided seeds for NIRS analysis: In total 1051 single seeds from different crosses segregating for oleic, linoleic and linolenic acid were scanned by NIRS (1340 –2000 nm) and subsequently analysed by gas liquid chromatography (GLC) for their fatty acid content. NIRS calibrations were developed using WinISI 1.61 software. Between 1014 and 1018 sample spectra were used for development of calibration equations.

External validation: In order to evaluate the performance of the calibration equations in routine analysis, cross validations and external validations have been accomplished. For external validations, spectra from seeds obtained from the four different breeding companies were separately removed from the primary calibration set and used as four different validation sets (V1-V4). New calibrations (C1-C4) were developed in each case from the remaining spectra. These calibrations were used to predict the fatty acid contents of the validation sets not represented in the calibration.

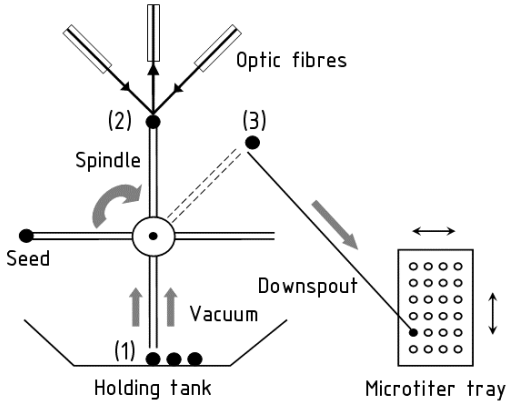

Figure 1. Principle of the automated sample presentation unit (PPM unum) for single rapeseeds constructed by Ingenieurbüro Steps (Jena) and VDLUFA (Kassel)

Results

In the complete calibration seed sample set the oleic acid content ranged from 43% to 89% with a standard deviation of 5.3% (Tab. 1). The standard error of cross validation (SECV) was 2.22 and the coefficient of determination in cross validation ($r(cv)^2$) was 0.81 (see also Fig. 2). The ratio SD/SECV was 2.4. Linoleic acid content ranged from 2.5% to 29% with a standard deviation of 4.8%. The SECV was 2.31 and the coefficient of determination in cross validation was 0.76. Linolenic acid content ranged from 1% to 13% with a standard deviation of 1.6. The SECV was 0.96 and the coefficient of determination in cross validation was 0.57. The analyses have been accomplished with a throughput of about 500 seeds per hour.

Table 1. NIRS calibration and cross validation statistics for the percentage of oleic *(18:1)*, linoleic *(18:2)* and linolenic acid *(18:3)* in the oil of intact single seeds of oilseed rape. Values as percentage of total fatty acids. *Mean*, standard deviation *(SD)* and *range* are GLC data. *SEC* standard error of calibration, *SECV* standard error of cross validation, $r(c)^2$ coefficient of determination in calibration, $r(cv)^2$ coefficient of determination in cross validation

Fatty acid	Mean	SD	Range			SEC	$r(c)^2$	SECV	$r(cv)^2$	SD/SECV
18:1	76.3	5.3	55.3	-	88.9	2.11	0.83	2.22	0.81	2.4
18:2	13.5	4.8	2.5	-	28.9	2.16	0.79	2.31	0.76	2.1
18:3	3.8	1.6	1.1	-	12.9	0.93	0.59	0.96	0.57	1.7

For external validation between 147 and 309 seed spectra were removed from the primary calibration set (Tab. 2). The calibrations developed with the remaining seed spectra showed for all three different fatty acids similar values for the SECV and for the coefficients of determination in cross validation; for comparison see Table 1 and Table 2. For all three fatty acids, the standard deviations of the four validations sets were lower compared to the primary calibration sets. For oleic and linoleic acid the standard errors of prediction corrected for the systematic error (SEP(C)) were in most cases much higher than the standard errors of calibration (SEC) of the primary calibration set. The SEP(C) was also large in comparison to the standard deviations of the validation sets. For oleic acid, the coefficients of determination in external validation ($r(e)^2$) ranged between 0.44 and 0.88 for the four different validation sets. For linoleic and linolenic acid the coefficients of determination in external validation ranged from 0.31 to 0.72 and from 0.11 to 0.33, respectively.

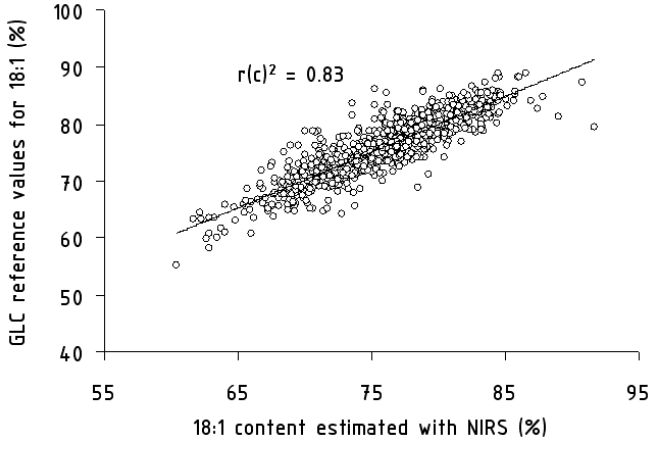

Figure 2. Relationship between oleic acid (18:1) content as determined by GLC and by NIRS

Table 2. NIRS external validation statistics for the percentage of oleic *(18:1)*, linoleic *(18:2)* and linolenic acid *(18:3)* in the oil of intact single seeds of oilseed rape. Values as percentage of total fatty acids. *Mean* and standard deviation *(SD)* are GLC data. *SEP*(C) standard error of prediction corrected by systematic error, $r(e)^2$ coefficient of determination in external validation

Fatty acid	Calibration			External Validation					
	Name	SECV	$r(cv)^2$	Name	N	Mean	SD	SEP(C)	$r(e)^2$
18:1	C1	2.2	0.82	V1	295	75.5	4.9	2.7	0.71
	C2	2.3	0.81	V2	309	80.4	4.1	3.5	0.44
	C3	1.9	0.86	V3	300	74.2	4.2	3.7	0.47
	C4	2.1	0.80	V4	147	76.0	4.6	2.9	0.88
18:2	C1	2.3	0.79	V1	295	14.6	4.3	2.9	0.60
	C2	2.6	0.73	V2	309	10.6	3.7	3.2	0.35
	C3	2.1	0.76	V3	300	14.0	3.4	3.9	0.31
	C4	2.1	0.76	V4	147	13.8	4.1	3.8	0.72
18:3	C1	1.0	0.51	V1	295	4.0	0.9	1.1	0.23
	C2	1.1	0.36	V2	309	2.6	0.6	0.9	0.14
	C3	0.9	0.60	V3	300	4.4	0.7	1.2	0.33
	C4	0.9	0.65	V4	147	3.9	0.8	2.0	0.11

Discussion

The preliminary results from this study show that the new high-throughput NIRS method can in principle be used to predict the oleic and linoleic acid content of single rapeseeds. However, the standard error in cross validation still appears high in comparison to the standard deviation (SD/SECV=2.4 and 2.1), indicating that an efficient selection will be possible only in F_2 seed populations showing a large variation (Fontaine et al. 2001). The comparison of the coefficient of determination in calibration, $r(c)^2$, with the coefficient of determination in cross validation, $r(cv)^2$, in Table 2 shows similar values, indicating a spectral homogenous seed population. Nevertheless, in external validation using different subsets of the primary calibration set, mostly inferior results were obtained, although calibration statistics (SECV, $r(cv)^2$) were similar. This indicates that the calibration set need to be extended by including more sample spectra from seeds derived from different crosses and different environments to become more robust in routine applications.

The development of a reliable calibration equation for linolenic acid showed to be more difficult. The SECV was high in comparison to the standard deviation (SD/SECV=1.7) and the results obtained in external validation show that a selection in routine applications will not be possible. Velasco et al. (1999) also reported inferior calibration statistics for linolenic acid in comparison to oleic acid, applying standard NIRS and single seed adapter ring cups. It needs to be shown whether the calibration can be improved and be made more robust by including more seed material from different crosses and environments.

Conclusions

The results obtained so far show that the new high-throughput NIRS technology is a promising method for the fast and non-destructive estimation of the fatty acid content of single seeds of oilseed rape. The analyses have been accomplished with a throughput of about 500 seeds per hour. However, it should be kept in mind that a considerable amount of time is also needed for handling, storage and checking the spectra for correct sorting, outliers, etc. More spectra from individual seed samples derived from different crosses and environments need to be included in the calibrations to make them more robust in validations and practical applications.

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References

Fontaine J, Hörr J, Schirmer B (2001) Near-infrared reflectance spectroscopy enables the fast and accurate prediction of the essential amino acid content in soy, repeseed meal, sunflower meal, peas, fishmeal, meat meal products, and poultry meal. J Agric Food Chem 49:57-66.

Möllers C (2004) Potential and future prospects for rapeseed oil. In: Gunstone FD (ed.): Rapeseed and Canola oil-production, processing, properties and uses. Blackwell Publishing, Oxford, UK, 186-217

Sato T, Uezono I, Morishita T, Tetsuka T (1998) Nondestructive estimation of fatty acid composition in seeds of *Brassica napus* L. by near-infrared spectroscopy. J Am Oil Chem Soc 75:1877-1881.

Velasco L, Möllers C, Becker HC (1999) Estimation of seed weight, oil content and fatty acid composition in intact single seeds of rapeseed (*Brassica napus* L.) by near-infrared reflectance spectroscopy. Euphytica 106:79-85.

Chemical composition of winter oilseed rape seeds in relation to the influence of nitrogen fertilisation and cultivar

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Abstract

The experiment was carried out in order to investigate the effect of nitrogen fertilisation on content of crude fat, total protein and glucosinolates in seeds and fatty acid composition in oil of five cultivars of oilseed rape. The effect of spring nitrogen fertilisation on qualitative characters was similar in all cultivars cultivated in the experiment. Small but significant effect of nitrogen fertilisation on total glucosinolate content in seeds was observed. Nitrogen fertilisation did not influence significantly fatty acid composition in oil. Environment conditions and interaction of environment conditions with agronomy and cultivar factors had not significant effect on qualitative characters evaluated in the experiment.

Key words: winter oilseed rape, nitrogen application, cultivars, glucosinolates, fatty acids.

Introduction

Quality of seeds is determined by cultivar genotype, environmental conditions and agronomical practices. Among agronomical practices the highest effect on quality of winter oilseed rape seeds has mineral fertilisation. Fat and protein content in winter oilseed rape seeds is significantly modified by spring nitrogen fertilisation (Muśnicki et al. 1999). The effect of nitrogen fertilisation on glucosinolate content in winter oilseed rape seeds (Wójtowicz et al. 2002, Wielebski and Wójtowicz 2004) and fatty acid composition (Delhaye and Guyot 1969, Kotecki et al. 2001, Muśnicki et al. 1999) in general is not significant. Nevertheless Bilsborrow P. E. et al. (1995) proved significant increase in glucosinolate content and Jędrzejak et al. (2005) showed variability of fatty acid composition under the influence of nitrogen fertilisation. Effect of fertilisation on quality of seeds can depend on environmental conditions. Also cultivars can react differently to nitrogen fertilisation level (Grate and Schweiger 1991).

The experiment was carried out in order to investigate the effect of nitrogen fertilisation on quality of seeds of five cultivars of oilseed rape.

Material and Methods

The subjects of laboratory investigation were seeds from two-year experiment performed in 2004 and 2005 in Łagiewniki Experimental Station situated in Greater Poland region. The experiment was conducted in split-plot design in four replications. The effect of nitrogen fertilisation applied in the form of ammonium nitrate (60, 100, 140, 180, 220 kg $N \cdot ha^{-1}$) to the content of crude fat, total protein and glucosinolates in seeds and fatty acid composition in oil of five cultivars of oilseed rape: Lisek - open pollinated variety, Mazur, Kaszub - composite hybrid varieties, BOH 3103, MR 153 – restored hybrid varieties, was investigated. Fat content was determined by Nuclear Magnetic Resonance. Protein content was estimated by Kjeldahl N determination x 6.25. Glucosinolate content and fatty acid composition was analysed using gas chromatography method.

Results and Discussion

Cultivars differed in crude fat and total protein content in seeds (Table 1). The smallest crude fat (44.4%) and total protein (18.0%) content were registered in seeds of cultivar Lisek. Seeds of Kaszub were characterised by the highest content of crude fat (46.0%). Cultivars did not differ in yield of fat and differences between protein yield were noticed only between restored hybrid cultivars.

Nitrogen fertilisation significantly influenced the content of crude fat and total protein in seeds. The increase of nitrogen dose from 60 to 220 $kg \cdot ha^{-1}$ caused the decrease of crude fat content from 46.0 to 44.2% and the increase of total protein from 18.4 to 20.1%. These results are consistent with previous work which demonstrated fat reduction and protein rise with an increase of nitrogen dose (Holmes and Ainsley 1978, Budzyński 1986, Wright et al. 1988, Taylor et al. 1991, Jasińska et al. 1993, Muśnicki et al. 1999, Wójtowicz et al. 2002).

The yield of fat and protein was dependent on nitrogen doses as well. The increase of fat yield was noticed up to the dose of 180 kg $N \cdot ha^{-1}$, and protein yield up to the dose of 220 kg $N \cdot ha^{-1}$.

The highest contents of total and alkenyl glucosinolate were registered in seeds of composite hybrid varieties: Kaszub and Mazur (16.4 and 14.6 $\mu M \cdot g^{-1}$ seeds) and the smallest restored hybrid varieties: BOH 3103 and MR 153 (10.8 and 9.0 $\mu M \cdot g^{-1}$ seeds) (Table 2). Lisek was characterised by the smallest content of indol glucosinolate in seeds (3.9 $\mu M \cdot g^{-1}$ seeds).

Nitrogen fertilisation had not significant effect on indol and alkenyl glucosinolate but influenced significantly total

glucosinolate content in seeds. The increase of nitrogen dose from 60 to 220 kg·ha^{-1} caused glucosinolate content increase from 12.0 to 13.2 μM·g^{-1}. Bilsborrow P. E. et al. (1995) suggest that nitrogen access to biosynthesis of glucosinolates increase with the growth of nitrogen fertilisation level.

Table 1. Influence of spring nitrogen fertilisation on content and yield of fat and protein (average for 2 years)

Factor		Content (%)		Yield (dt·ha^{-1})	
		crude fat	total protein	crude fat	total protein
Cultivar	Lisek	44.4 a*	18.0 a	25.8 a	10.5 ab
	Mazur	45.0 ab	19.5 b	25.0 a	10.9 ab
	Kaszub	46.0 c	19.3 b	24.9 a	10.5 ab
	BOH 3103	45.7 bc	19.3 b	24.2 a	10.2 a
	MR 153	45.1 ab	19.5 b	25.3 a	11.0 b
$LSD_{0.05}$		0.78	0.61	ns**	0.73
Nitrogen dose (kg·ha^{-1})	60	46.0 c	18.4 a	23.7 a	9.5 a
	100	45.7 bc	18.6 a	24.7 ab	10.1 ab
	140	45.3 bc	19.0 ab	25.5 b	10.7 bc
	180	44.9 ab	19.6 bc	25.8 b	11.2 c
	220	44.2 a	20.1 c	25.4 b	11.5 c
$LSD_{0.05}$		0,95	0.91	1.68	1.07

*numbers in columns marked with the same letters did not differ significantly
**ns – no significant difference

Table 2. Influence of spring nitrogen fertilisation and cultivar on glucosinolate content in seeds (average for 2 years)

Factor		Indol glucosinolate content (μM·g^{-1} of seeds)	Alkenyl glucosinolate content (μM·g^{-1} of seeds)	Total glucosinolate content (μM·g^{-1} of seeds)
Cultivar	Lisek	3.9 a	7.7 c	11.6 c
	Mazur	4.6 b	10.0 d	14.6 d
	Kaszub	4.8 b	11.7 e	16.4 e
	BOH 3103	4.8 b	6.0 b	10.8 b
	MR 153	4.8 b	4.2 a	9.0 a
$LSD_{0.05}$		0.38	1.36	1.37
Nitrogen dose (kg·ha^{-1})	60	4.4	7.6	12.0 a
	100	4.6	7.5	12.1 ab
	140	4.4	7.7	12.1 ab
	180	4.6	8.3	13.0 ab
	220	4.8	8.4	13.2 b
$LSD_{0.05}$		ns	ns	1.12

Explanations below Table 1

Seeds of cultivars investigated in the experiment differed in fatty acid composition in oil (Table 3). Significant differences referred to the content of stearic, oleic, linolenic, eicosenic acid and PUFA (linoleic and linolenic). Nitrogen fertilisation had not significant effect on fatty acid composition in oil. Nevertheless small decrease of oleic acid and increase of PUFA was noticed when higher nitrogen doses (180 and 220 kg·ha^{-1}) were applied.

Table 3. Influence of spring nitrogen fertilisation and cultivar on fatty acid composition in winter oilseed rape oil (%) (average for 2 years)

$C_{16:0}$ – palmitic
$C_{18:0}$ – stearic
$C_{18:1}$ – oleic
$C_{18:2}$ – linoleic
$C_{18:3}$ – linolenic
$C_{20:1}$ – eicosenic
$C_{22:1}$ – erucic

Factor		Fatty acid composition (%)									
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	$C_{22:1}$	$C_{18:1}+C_{18:2}+C_{18:3}$	$C_{18:2}+C_{18:3}$	$C_{18:2}/C_{18:3}$
Cultivar	Kaszub	4.69 a	1.74 a	61.36 abc	20.40 ab	10.02 b	1.54 ab	0.26 a	91.77 a	30.42 b	2.04 b
	Lisek	4.70 a	1.87 b	62.56 d	19.67 a	9.46 a	1.52 ab	0.26 a	91.68 a	29.12 a	2.08 c
	Mazur	4.65 a	1.73 a	60.89 ab	20.39 ab	10.25 b	1.72 b	0.37 a	91.52 a	30.63 bc	1.99 a
	BOH 3103	4.71 a	1.88 b	61.70 cd	20.47 b	9.68 a	1.42 a	0.14 a	91.84 a	30.14 b	2.11 d
	MR 153	4.67 a	1.73 a	60.55 a	21.38 c	9.98 b	1.48 a	0.22 a	91.91 a	31.36 c	2.14 e
$LSD_{0.05}$		ns	0.052	0.927	0.753	0.291	0.230	ns	ns	0.913	0.014
Nitrogen dose (kg·ha^{-1})	60	4.73 a	1.79 a	61.50 a	20.37 a	9.80 a	1.52 a	0.28 a	91.67 a	30.18 a	2.08 a
	100	4.69 a	1.80 a	61.59 a	20.27 a	9.86 a	1.51 a	0.30 a	91.72 a	30.13 a	2.06 a
	140	4.63 a	1.77 a	61.70 a	20.36 a	9.82 a	1.50 a	0.23 a	91.88 a	30.18 a	2.07 a
	180	4.69 a	1.80 a	61.20 a	20.67 a	9.96 a	1.54 a	0.17 a	91.83 a	30.63 a	2.08 a
	220	4.69 a	1.81 a	61.06 a	20.64 a	9.93 a	1.61 a	0.28 a	91.63 a	30.56 a	2.08 a
$LSD_{0.05}$		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Explanations below Table 1However, Jędrzejak et al. (2005) on the basis of the experiment with spring rape, showed significant growth of palmitic, stearic, linoleic and linolenic acid and reduction of oleic acid under the influence of nitrogen fertilisation increase. This dependence likely resulted from nitrogen fertilisation effect on the rate of seed ripening.

Bartkowiak-Broda and Krzymański (1981) showed that during seed ripening palmitic, stearic, linoleic, linolenic acids decreased and intensive oleic acid accumulation took place.

Conclusions

The effect of spring nitrogen fertilisation on investigated quality features was similar in all cultivars evaluated in the experiment. The increase of nitrogen dose from 60 to 220 kg·ha^{-1} caused the decrease of crude fat content from 46.0 to 44.2% and the increase of total protein from 18.4 to 20.1% and glucosinolate from 12.0 to 13.2 μM·g^{-1}.

In investigated cultivars the increase of fat yield was noticed up to the dose of 180 N·ha^{-1}, and protein yield to the dose of 220 kg N·ha^{-1}.

The cultivars differed in crude fat and total protein content and fatty acid composition. Seeds of cultivar Kaszub were characterised by the highest content of fat and glucosinolate. The smallest content of fat, protein and indol glucosinolate were noticed in seeds of cultivar Lisek.

Small but significant differences referred to the ratio of linoleic to linolenic acid content, which ranged from 1.99 in cultivar Mazur to 2.14 in cultivar MR 153.

References

Bartkowiak-Broda I., Krzymański J. 1981. Zmiany w składzie chemicznym nasion ozimego rzepaku bezerukowego K-2040 w czasie formowania i dojrzewania. Biuletyn IHAR, 146: 25-33.

Bilsborrow P. E., Evans E. J., Zhao F. J. 1995. Changes in the individual glucisinolate profile of double low oilseed rape as influenced by spring nitrogen application. Proc. 9th Int. Rapeseed Congress. Cambridge, UK. (2): 553-555.

Budzyński W. 1986. Studium nad wpływem niektórych czynników agrotechnicznych na zimowanie i plonowanie odmian podwójnie uszlachetninych rzepaku ozimego. Acta Acad.Agricult. Tech. Olst., Agricult., 41, suppl, B:1-56.

Delhaye R., Guyot A. 1969. Etude par chromatographie gazeuse des huiles extraites de graines de queques varietes de colza d'hiver recoltees au Centre de Recherches Agronomiques de Gembloux. Bull. Inst. Agron. Gembloux, 4, 1: 44:65.

Gerath H., Schweiger W. 1991. Improvement of the use of nutrients in winter rape - a strategy of economically and ecologically responsible fertilizing. Proc. 8 th Intern. Rapeseed Congress, Saskatoon, 4: 1197-1201.

Holmes, M. R. J., and Ainsley, A. M. 1978. Seedbed fertilizer requirements of winter oilseed rape. J. Sc. Fd. Agric., 29: 657-666.

Jasińska Z., Malarz W., Budzyński W., Toboła P. 1993. Wpływ sposobu wiosennego nawożenia azotem na plonowanie rzepaku ozimego. Post. Nauk Roln., 6: 33-40.

Jędrzejak M., Kotecki A., Kozak M., Malarz W. 2004. II. Wpływ zróżnicowanych dawek azotu na profil kwasów tłuszczowych oleju rzepaku jarego. Rośliny Oleiste – Oilseed Crops, XXVI (1): 139-149.

Kotecki A., Kozak M., Malarz W., Aniołowski K. 2001. Wpływ nawożenia azotem na skład chemiczny nasion pieciu odmian rzepaku jarego. Rośliny Oleiste – Oilseed Crops, XXII (1): 81-89.

Muśnicki Cz. Toboła P., Muśnicka B. 1999. Wpływ niektórych czynników agrotechnicznych i siedliskowych na jakość plonu rzepaku ozimego. Rośliny Oleiste – Oilseed Crops, XX (2): 459-469.

Taylor A. J., Smith C. J., Wilson I. B. 1991. Effect of irrigation and nitrogen fertilizer on yield, oil content, nitrogen accumulation and water use of canola (*Brassica napus L.*). Fertilizer Res., 29: 249-260.

Wright G. C., Smith C. J., Woodroofe M., R. 1988. The effect of irrigation and nitrogen fertilizer on rapeseed *(Brassica napus L.)* production in south-eastern Australia. I. Growth and seed yield. Irrig. Sci., 9: 1-13.

Wielebski F., Wójtowicz M. 2004. Wpływ czynników agrotechnicznych na skład chemiczny nasion odmiany mieszańcowej zrestorowanej w porównaniu z odmianą populacyjną i odmianami mieszańcowymi złożonymi. Rośliny Oleiste – Oilseed Crops, XXV (2): 505-519.

Wójtowicz M, Wielebski F, Czernik-Kołodziej K. 2002. Wpływ wiosennego nawożenie azotem na cechy rolnicze i użytkowe nowych form hodowlanych rzepaku ozimego. Rośliny Oleiste – Oilseed Crops, XXIII (2): 337-350.

Image analysis of mustard seed: its utilization in assessing seed uniformity

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Abstract

Mustard millers have long had a strong interest in obtaining uniform seed. They prefer mustard seed to be all the same colour. Oriental and yellow mustard should be uniformly bright yellow and brown mustard should be dark brown. Admixtures of colors are not tolerated. Secondly, to ease milling difficulties the seed should be, as nearly as possible, all the same size and shape (like ball bearings). Reflective colorimeters can assess seed color and predict which seed sample is the brightest yellow or the darkest brown but they fall short in assessing how uniform the seed color is among seeds or within individual seeds (mottling). 1000 seed weight is used to estimate the average seed size of a sample, but it does not provide information on uniformity of seed size. To do this individual seed weights of many seeds per sample have to be recorded - a tedious and time-consuming task. Visual observation can provide a rough estimate of seed color, size and shape, but is a subjective measurement. With the advent of digital cameras and image processing software it is now possible to routinely assess seed uniformity. A Canon EOS Digital Rebel was utilized to photograph 300 seed samples of seed harvested from several locations and years of the Western Canada co-operative mustard trial. The photographs were then processed with the public domain program "ImageJ". The "Analyze Particles…" option was used to calculate seed size, shape and shade data for each seed of each sample which was used to generate size, shape and shade uniformity statistics. With this setup up to 200 samples could be photographed and processed each day per technician. It was found that there were consistent differences in seed uniformity among the different cultivars and lines in each category of mustard, suggesting that there were genetic differences between lines which could be capitalized on in future breeding efforts.

Key words: Mustard, *Brassica juncea*, digital image analysis, seed size, seed shape, seed shade, ImageJ

Introduction

The mustard trade has strict regulations with respect to cleanliness, allowing not more than 0.7% extraneous material and not more than 2% damaged or shrivelled seed (Hemingway 1995). The seed coat is a significant, variable portion of *Brassica juncea* and *Sinapis alba* seeds, and its proportion influences the economics of dry-milling where the seed coats are removed, and wet-milling of *B. juncea* where the seed coat is left in or a portion screened out. Seed shape and uniformity of size are important to maximizing seed coat separation/milling (Hemingway 1995, 1997). Also the tendency of seed coats to break into tiny fragments affects the quality of mustard (Hemingway 1995). Appearance is very important to the mustard, thus oriental mustard and yellow mustard seed should be a bright yellow with no dark seeds. Brown mustard seed should be dark brown in colour with no light coloured seeds. As our centre (Saskatoon Research Centre) is the primary breeding institution for condiment mustard, the Canadian Mustard Association has asked us to develop highly uniform cultivars of oriental, brown and yellow mustard in terms of seed size, seed shape (as round as possible) and seed colour. For breeding applications methods of analysis must be accurate and, importantly, rapid and it seemed that digital image analysis could be an ideal method to approach this problem. A digital camera and the free scientific image processing program, ImageJ (Abramoff et al. 2004, Rasband 1997-2006) are the essential tools to approach this task. Others have used image analysis to assess visual characteristics. Cober et al. 1997 studied the heritability of seed shape and size in soybeans, Dell'Aquilla 1997 and Ducournau et al. 2004 used image analysis to monitor germination of broccoli seeds and sunflowers, respectively, and Tanska et al. 2005 studied surface features and colour of rapeseed. Others have used image analysis for seed classification purposes (Utku et al. 1998, Wrigley 1999, Sako et al. 2001).

Material and Methods

Digital imaging hardware: The imaging hardware consists of a Canon EOS Digital Rebel camera (6 megapixel sensor; EF-S 18-55mm f/3,5-5,6 USM lens,), an IFF Mini-Repro copy stand with 4 lights and a transparency viewer (GEPE 8" x 12" Slimlight Illuminator). The camera is mounted on the copy stand post 30 cm above the slide viewer mounted on the base. The camera settings are auto focus, M, ISO 100, optical zoom 35, aperture 16, shutter 1/10, exposure 0 sharp 0, and white balance: Fluorescent. Images are captured in large/fine JPG format to a database of pictures (Microsoft Access). The camera settings are not changed throughout and a picture of a ruler is used to scale the seed sample images. Total cost of the equipment was less than $2000 Canadian.

Seed material and seed sample preparation: The seed material used for this study came from the Western Canada co-operative mustard trials conducted each year at several locations across the prairies. Seeds are sampled from harvest envelopes using a 300 seed vacuum seed counting head (Hoffman Manufacturing, Inc., Albany, OR, USA). They are placed on a 20 x 25 cm glass plate which has been lightly sprayed with Krylon® Easy-Tack™ repositionable spray adhesive by

placing the seed head upside down and releasing the vacuum. The adhesive is essentially invisible to the camera and holds the seeds in place. Plastic film is positioned at the top and bottom of the glass plate such that it catches the top and bottom edge of the seed head and keeps it just slightly off the surface. The plate is positioned on the image viewer and a picture is taken and stored in the database. The seeds are removed by scraping them into a container using a straight edge and the plate is reused. The adhesive treatment typically lasts for about 50 – 200 images before it becomes so soiled that it interferes with image quality. The glue is easily washed off with soapy water and reapplied. In this manner about 100 to 200 seed sample images are created per day.

Image Processing: The particle analyzing option of the freeware image processing program, "ImageJ" (Rasband 1997-2006) is used to generate descriptive statistics for individual seeds of the sample and of the entire population. A small plugin (a modification of the Batch Converter plugin, Rasband 1997-2006) was created to allow automatic processing of images in a folder with generation of particle/seed statistics for each image. The images are converted to 8-bit greyscale, a threshold of 0-180 is applied and several statistics are generated for each seed in the image including area, mean grey value, standard deviation of grey value, perimeter, major and minor axis, circularity, Feret's diameter, etc. The batch program processes about 100 pictures in 5 minutes. This data is stored in the database where it can be manipulated as desired.

Results

A typical image of 300 seeds is shown in Fig. 1a. As the image size is 3072 x 2048 pixels each seed is represented by greater than 500 pixels. Different shapes, sizes and colours of seed can be readily seen. Also it is evident that some seed are broken and some are germinated. All seeds are cleanly separated which aids the software in discrimination. A comparison of Fig. 1a and 1c with their threshold counterparts (Fig. 1b and 1d) reveals that the threshold (red) closely follows the outlines of the individual seeds and completely covers the seed, so accurate estimates of seed size, shape and shade can be obtained.

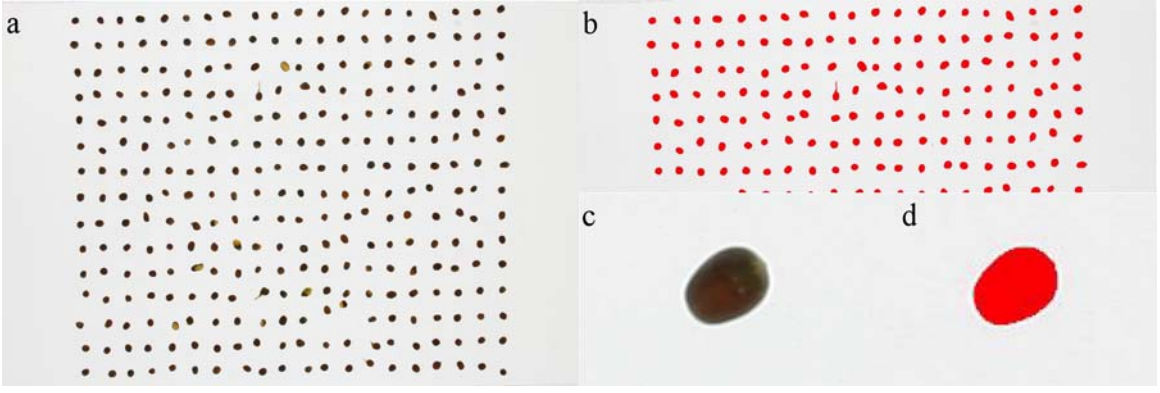

Fig.1 Image of 300 seeds of brown mustard, a line with poor seed quality (a: original picture; b: image top half, 8-bit greyscale conversion and threshold applied; c: a single seed; d: same seed, threshold applied).

Values for area, diameter and greyness generated for each seed can be averaged and compared to traditional methods estimating seed mass (1000 seed weight) and colour (whiteness index generated by a Hunterlab Miniscan colorimeter). Fig. 2a compares image analysis average area data with 1000 seed weight. A strong correlation was found (R^2 = 0.966). Fig. 2b demonstrates that diameter also correlates strongly with 1000 seed weight (R^2 = 0.962), as expected. Fig. 2c shows that average mean grey value can be used to estimate seed brightness, the same as the whiteness index.

Table 1 compares three oriental mustard entries in the 2005 cooperative mustard trial for seed characteristics that can be determined by digital image analysis. There is a consistent difference across locations in average seed area between Forge (a small seeded cultivar), AC Vulcan and J01-1429 (a line selected for increased seed mass). The standard deviation of area (uniformity of seed size) is also consistently higher in J01-1429 than Forge and AC Vulcan. In circularity (a measure of roundness) and in standard deviation of circularity Forge is consistently the closest to a round shape and has the least variation of shape (both desirable traits, Hemingway 1995, 1997). In mean grey value AC Vulcan is better than Forge and J01-1429. But there is no clear winner for uniformity of shade (standard deviation of mean grey value).

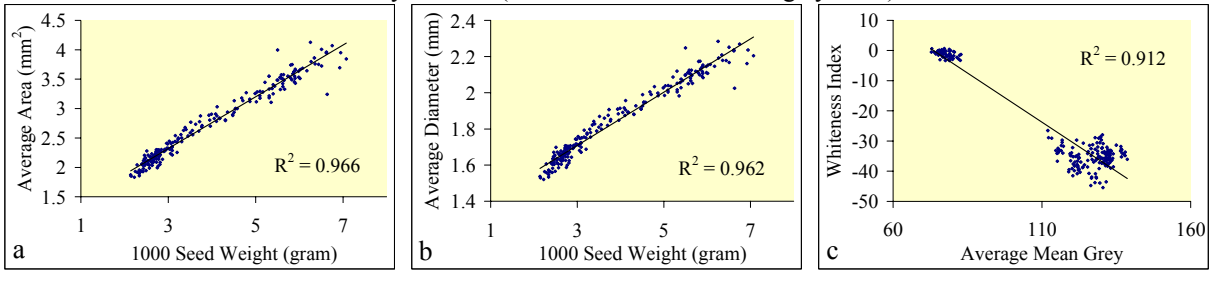

Fig. 2. 2005 Cooperative mustard trial, correlation of ImageJ values with standard methods of estimating seed mass and colour (a: ImageJ average area versus 1000 seed weight, b: ImageJ average diameter versus 1000 seed weight, c: ImageJ average grey value versus whiteness index).

Table 1: Seed uniformity comparisons of three oriental mustard entries in 2005 cooperative mustard trial

Entry	Location*							
	C1	E2	I0	R1	S1	S6	W1	Avg
Area (mm^2)								
Forge	1.90	2.08	2.12	2.05	2.15	1.82	2.10	2.04
AC Vulcan	2.07	2.33	2.27	2.31	2.38	2.01	2.28	2.24
J01-1429	2.71	2.86	2.71	2.80	3.00	2.56	2.85	2.79
SD Area (mm^2)								
Forge	0.30	0.38	0.38	0.35	0.35	0.34	0.37	0.35
AC Vulcan	0.28	0.37	0.34	0.41	0.35	0.33	0.34	0.35
J01-1429	0.51	0.50	0.42	0.52	0.54	0.45	0.48	0.49
Rel. SD Area (%)								
Forge	15.8	18.5	17.7	16.8	16.1	18.5	17.6	17.3
AC Vulcan	13.8	15.8	15.1	17.8	14.5	16.4	14.8	15.4
J01-1429	18.7	17.4	15.6	18.4	18.0	17.7	17.0	17.6
Circularity								
Forge	0.908	0.893	0.896	0.903	0.901	0.903	0.895	0.900
AC Vulcan	0.902	0.888	0.889	0.878	0.895	0.895	0.887	0.890
J01-1429	0.895	0.883	0.891	0.890	0.890	0.894	0.885	0.890
SD Circularity								
Forge	0.015	0.036	0.024	0.028	0.024	0.020	0.034	0.026
AC Vulcan	0.025	0.035	0.025	0.064	0.022	0.018	0.038	0.033
J01-1429	0.034	0.041	0.024	0.038	0.035	0.022	0.034	0.033
Mean Grey Value								
Forge	122.8	121.0	115.3	116.7	116.9	123.6	120.5	119.4
AC Vulcan	129.5	127.0	121.6	122.0	123.2	130.0	128.1	125.8
J01-1429	122.6	119.2	115.5	115.0	116.5	121.3	121.4	118.5
SD Mean Grey								
Forge	7.2	7.3	12.3	9.0	8.4	5.8	9.3	8.6
AC Vulcan	8.1	7.0	12.4	10.9	9.5	5.6	7.4	8.8
J01-1429	8.2	8.2	12.4	11.1	9.4	6.1	7.7	9.2

* Locations: (C1 – Congress AB, E2 – Eyebrow SK, I0 – Irricana AB, R1 – Rosebank MB, S1 – Saskatoon SK, S6 –Swift Current SK, W1 Weyburn SK), Avg – average of all locations, Area – average area, Circularity – (A measure of roundness, 4*pi*area/perimeter^2, 1 is a circle, 0 is a line), Mean Grey Value – average greyness (a higher number is brighter), SD – standard deviation.

Discussion

The data for only three lines of oriental mustard in 2005 is shown. But co-operative mustard image data from 2002, 2003 and 2004 is available as well. The observations described here are consistent with the observations made in previous years. Forge is consistently the smallest, but is the roundest and the most uniform in terms of size and shape. AC Vulcan has consistently larger seed than Forge and is a brighter seed, but is not quite as round. Similar observations of differences in seed size, shape, colour and uniformity have been observed amongst cultivars of brown mustard and yellow mustard as well.

This method is also used to conduct selections within breeding populations of mustard and is useful for quantifying the susceptibility of certain lines to seed damage and early germination. These events drastically alter the shape of the seed and thus manifest themselves as a reduction in the circularity value. These events also drastically affect the color or mean gray value, as an emerging radical or the visible meat of a seed is significantly brighter than the brightest intact oriental mustard seed.

Conclusions

Digital image analysis is a useful tool for rapid screening of a large number of samples for seed size, shape and shade parameters including estimates of the uniformity of these seed characteristics. Small differences are detectable. The "ImageJ" software program is cost-effective (free), powerful and easily adaptable and is recommended for anyone on a budget. Different cultivars of mustard differ in their seed size, seed shape (some lines are more round or circular) and colour, but by this image analysis method it can be seen that different lines of mustard also differ with respect to uniformity of seed size and shape. The equipment and software needed to conduct digital image analysis is relatively inexpensive and many seed samples can be processed in a single day, up to 200.

References

Abramoff M.D., Magelhaes P.J. Ram, S.J. (2004). Image processing with ImageJ. Biophotonics International, **11**, 36-42.
Cober E.R., Voldeng H.D., Fregeau-Reid J.A. (1997). Heritability of seed shape and seed size in soybean. Crop Science **37**, 1767-1769.

Dell'Aquila A. (2005). The use of image analysis to monitor the germination of seeds of broccoli (*Brassica oleracea*) and radish (*Raphanus sativus*). Annals of Applied Biology **146**, 545-550.

Ducournau S., Feutry A., Plainchault P., Revollon P., Vigouroux B., Wagner M.H. (2004). An image acquisition system for automated monitoring of the germination rate of sunflower seeds. Computers and Electronics in Agriculture **44**, 189-202.

Hemingway J.S. (1995). The Mustard Species: Condiment and Food Ingredient Use and Potential as Oilseed Crops. In *Brassica* Oilseeds Production and Utilization, ed. Kimber D.S., McGregor D.I., CAB International Chapter **17**, 373-383.

Hemingway J.S. (1997). Advantageous choice of mustard varieties for processing. http://www.mancan.mb.ca/jhemway2.html

Rasband W.S., (1997-2006). ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/

Sako Y., Regnier E.E., Daoust T., Fujimura K., Harrison S.K., McDonald M.B. (2001). Computer image analysis and classification of giant ragweed seeds. Weed Science **49**, 738-745.

Tanska M., Rotkiewicz D., Kozirok W., Konopka I. (2005). Measurement of the geometrical features and surface color of rapeseeds using digital image analysis. Food Research International **38**, 741-750.

Utku H., Koksel H., Kayhan S. (1998). Classification of wheat grains by digital image analysis using statistical filters. Euphytica **100**, 171-178.

Wrigley C.W. (1999). Potential methodologies and strategies for the rapid assessment of feed-grain quality. Aust. J. Agric. Res. **50**, 789-805.

Screening of Indian mustard genotypes for heavy metal accumulation tendency under in-vitro conditions

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Abstract

Indian mustard (*Brassica juncea* L.Czern. & Coss.) have been established as a potential phytoremediator for heavy metals. It may be due to its inherent metal tolerance genes, which is not yet been identified. Heavy metal contamination of soils is increasing nowadays. Cadmium (Cd) and nickel (Ni) are some of the examples for heavy metals found in the soil. The ability of Indian mustard genotypes to accumulate Cd and Ni was assessed under controlled conditions. The soils were artificially contaminated by 3 and 5mM metal concentrations of both cadmium acetate and nickel sulphate with respect to control. Shoot length, root length, fresh weight and dry weight of genotypes were analyzed at 60, 90 and 105 days after germination. Heavy metal accumulation (Both roots and shoots) and yield attributes at the time of harvesting were also analyzed. Our results showed significant difference in the case of root length, shoot length, fresh weight and dry weight between Cd and Ni treatments. Exogenous supply of 3mM Cd and Ni caused less reduction in root and shoot length in most of the genotypes whereas at higher doses (5mM) caused more reduction. At a stress dose of 5mM, the nickel accumulation in the shoots and roots was on average of 16.1, 0.75µgm/gm dry weight respectively. For the same stress dose, cadmium accumulation in shoots and roots was an average of 31.40µgm/gm and 12.71µgm/gm dry weight respectively. In both the cases shoots accumulated more amount of heavy metals compared to roots. It is, therefore, *Brassica juncea* offers better accumulation potential for Cd compared to Ni.

Key words: Indian mustard, phytoextraction, nickel, cadmium

Introduction

Metals are intrinsic components of earth's crust. Heavy metals are the stable metals or metalloids whose density is greater than 5g/cm ^{3}, namely lead (Pb), copper (Cu), nickel (Ni), cadmium (Cd) and mercury (Hg). The heavy metal contamination has increased in urban vicinities due to rapid industrialization and ill environmental management (Wagner, 1993). The presence of heavy metals in agricultural ecosystem has raised concern not only for crop quality but also for human health. There is an increasing concern about the accumulation of Cd and Ni in food chains. Commonly used methods dealing with heavy metal pollution are either the extremely costly process of excavation and burial or simply isolation of the contaminated sites. But in recent years, researchers have observed fast growing, biomass accumulating plants including agronomic crops for the ability to tolerate and accumulate metals in their roots and shoots as a detoxification mechanism (Mkansdawire et al., 2004; Chaney et al., 2005; Chang et al., 2005; Fayiga and Ma, 2006). *Brassica juncea* is a high biomass crop, which has a potential for bioremediation of heavy metals like Cd (Singh et al., 2001; Quadir et al., 2004) and Ni (Panwar et al., 2002; Kerked and Kramer, 2003). Recently transgenic Indian mustard over expressing gamma glutamine cysteine synthetase (Schafer et al., 1998; Zhu et al., 1999) have been reported as potential hyperacculator and can be used as a viable cost effective alternative to clean up metal contaminated soils. In this study the ability of Indian mustard (*Brassica juncea*) to uptake Cd and Ni was assessed under controlled conditions.

Materials and methods

Ten different varieties of Indian mustard (B-350, CSR-94, CSR-931, DWDR-486, EC-223389, IC-94280, NDR-873, PR-45, RWPC-10 and RW-2Z) were received from IARI, Pusa, New Delhi. These experiments were conducted at the controlled conditions. Initially about 25 seeds were planted in each pot of 12" height with 8" bottom. Each pot contained about 10 kg of soil and supplied initially with basal dose of N, P, K and S and second dose of N and S after 45-day of sowing. These pots were watered with 3.0 and 5.0mM Cd^{2+} salt and Ni $^{2+}$ salt dissolved in half strength Hoagland solution, first as basal dose, and second after 7 days of sowing i.e., just after emergence of the seedlings from the pots. 100ml of half strength Hoagland solutions was used to irrigate all the pots after 30 days of sowing and thereafter on every 15 days interval to maintain the soil nutrient mineral ratios in pots. Thinning of all the pots was done after 15 days of sowing and 10 plants in each pot were retained. Growth of the plants was measured after 60, 90,105 days of sowing. The fresh and dry weight were recorded at 60 and 125 days after seedling emergence. At the maturity of crop the yield attributes of the genotypes were determined in terms of number of pods per plant, number of seeds per pod, weight of 1000 seeds and total yield per plant. The metal accumulation in different plant parts was also determined on atomic absorption spectrophotometer after digestion in a mixture of nitric-perchloric acid and expressed on the dry matter basis. Data was analyzed statistically using ANOVA.

Results

Exogenous supply of 3mM Cd and Ni caused less reduction in root and shoot length where as 5mM stress caused more

inhibition in growth that is evident from the Figure 1. Decrease noticed in root length was in the range of 19.5 % for 5mM Cd and 38.47% at 5mM Ni for 105 days after exposure. Shoot length was inhibited by 10.67% and 28.39% compared to the control when analyzed after 105 days of exposure to 5mM stress doses of Cd and Ni respectively. Figure 2 shows the effect on fresh and dry weights of the *Brassica juncea* genotypes after 60 and 125 days after exposure of heavy metals. In comparison to control, all the genotypes exhibited sensitivity to Ni and showed highly significant ($p \leqslant 0.05$) reduction in fresh weight and dry weight in the late growth periods. Fresh weight and dry weight of the genotypes reduced by 54.64% and 46.12% respectively after 125 days of Ni stress dose. The heavy metal Cd at the lower concentrations increased the fresh weight (13.11%) and dry weight (18.85%) compared to the fresh and dry weight of the control when analyzed after 125 days of exposure. An increasing Cd supply markedly reduced the fresh and dry weight of genotypes 125 old *B.juncea* genotypes supplemented with 5mM Cd are capable of accumulating 12.71µgm/gm of their roots and between 31.40µgm/gm of their shoots on a dry weight basis. Metal accumulated under a stress of 5mM Ni was 0.75 µgm/gm in roots and 16.11µgm/gm in shoots respectively. All Brassica genotypes showed much higher Cd accumulation in both roots and shoots at both concentrations compared to Ni accumulation under similar conditions which is evident from the data (Table 1). The results indicate that *Brassica juncea* differ significantly in Ni and Cd shoot root ratio and showed higher phytoextraction potential for Ni compared to Cd. At the completion of crop growth we analyzed yield attributes of the genotypes in terms of number of pods per plant, number of seeds per pod, weight of 1000 seeds, yield per plant (Table 2).

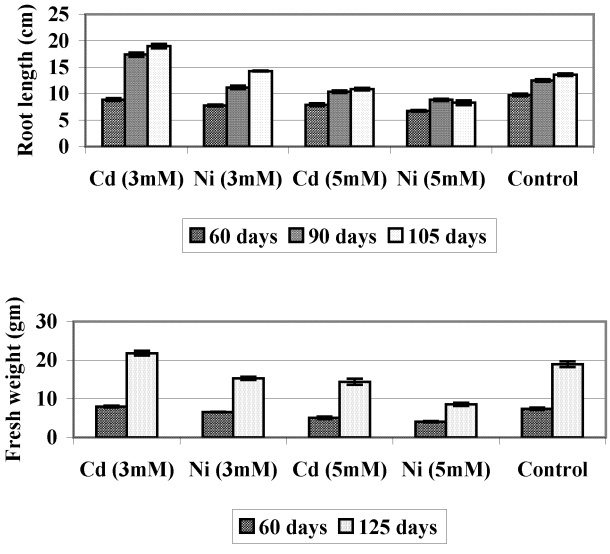

Fig. 1. The effect of Cd and Ni on root length and shoot length of *B.juncea* at different stress doses (3 &5mM) and at different days after exposure. The data represent means ± SE. Vertical bars represent SE.

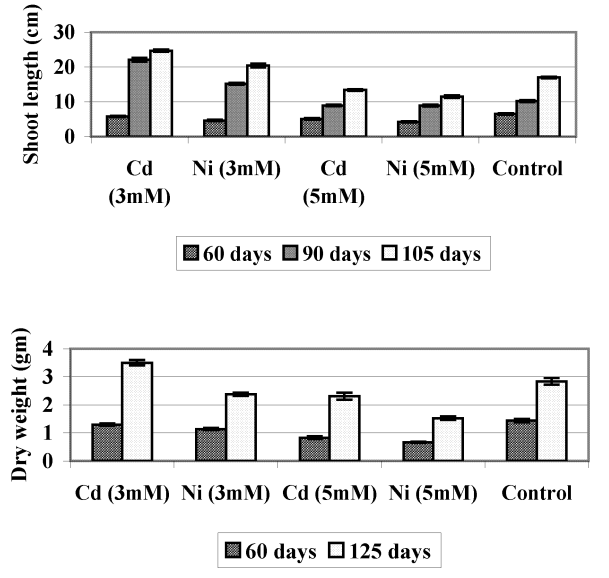

Fig. 2. The effect of Cd and Ni on fresh weight and dry weight of *B.juncea* at different stress doses (3 &5mM) and at different days after exposure. The data represent means ± SE. Vertical bars represent SE.

Table 1. Heavy metal accumulation and shoot / root ratio (S/R ratio) in Indian mustard genotypes grown in pots for 125 days with 3 and 5mM stress doses of cadmium acetate and nickel sulphate.

	Roots (µg/g) dry weight	Shoots (µg/g) dry weight	S/R ratio
3 mM Cd	5.93 (*0.2912*)	24.20 (*0.9163*)	4.06
5mM Cd	12.71 (*0.2762*)	31.40 (*0.6856*)	2.47
3mM Ni	0.27 (*0.0267*)	9.32 (*0.3021*)	35.86
5mM Ni	8.8 (*0.0240*)	16.11 (*0.3196*)	21.46

Mean of five replicates: in brackets: standard error ($p \leqslant 0.05$)

Table: 2 Yield Attributes of *B.juncea* genotypes after heavy metal exposure

	Control	Cadmium	Nickel
No of pods per plant [a]	72.88 (*4.700*)	68.53 (*2.017*)	59.77 (*0.7412*)
No of seeds per pod [b]	6.77 (*0.3239*)	5.88 (*0.9279*)	7.11 (*0.3093*)
Weight of 1000 seeds [c]	3.67 (*0.0837*)	3.28 (*0.0394*)	2.57 (*0.0570*)
Yield per plant [d]	1.77 (*0.1169*)	1.28 (*0.0768*)	1.08 (*0.0579*)

[a] Mean of 10 plant replicates [b]Mean of 1000 pods [c]Mean of 5 replicates [d]Mean of 10 plants
: in brackets standard error ($p \leqslant 0.05$)

Discussion

Heavy metal addition significantly reduced the root length and shoot length of *Brassica juncea* genotypes. A decrease in root and shoot length was observed in rice plants when grown for ten days in a medium containing these heavy metals (Rubio et al*.,* 1994). Ozturk et al., (2003) found similar affects using Cd in wheat cultivars. Recently Su et al., (2004) showed that Cd stress significantly reduced the root volume and length of oil seed rape and Indian mustard. It was found that heavy metals affect root growth more severely than shoot growth. The results of previous studies showed that Cd affects root growth more severely than shoot growth (Vaselov et al., 2003). Significant difference was found between the Cd and Ni exposure for the fresh and dry weight parameters. The Cd exposure significantly reduced the dry and fresh weight of roots and shoots of *B.campestris* (Zhu et al., 2004). The heavy metal concentration in the roots and shoots increased with increased heavy metal concentration in the soil and the relationship between two concentrations are similar to all genotypes. The accumulation of heavy metals like Cd (Singh and Brar, 2002) and Ni (Panwar et al., 2002) by *Brassica juncea* increased with increasing their levels in the soil. Wang and Su (2005) reported that the amount of Cd uptake increased with the increase of growth period of Indian mustard. Accumulation of Ni was found to be more in shoots than in roots. Plants that hyperaccumulate Ni exihibit an exceptional degree of Ni tolerance and the ability to translocate Ni in large amounts from root to shoot (Ingle et al*.,* 2005; Dan et al*.,* 2002). Increase in concentration of Ni (3mM to 5mM) did not change appreciably its content in roots but shoot accumulation showed considerable increase at higher dose of Ni in soil. All the genotypes accumulated Cd and Ni in their shoots more than their roots. Plants can extract Cd from the soil and transport it via the xylem in to shoots and leaves where it can accumulate (Blaylock et al., 2000). The ability of plants to accumulate metal in the shoots is important because the shoots represent the harvestable biomass. The higher relative shoot accumulation will result in a higher S/R ratio. The species able to accumulate relatively high metal concentration in above ground tissues would be the good candidate for phytoextraction (Dang et al., 2004). Plant potential for Cd extraction generally depends on shoot Cd concentration (Vassilev, 2002). Therefore, *B. juncea* genotypes exhibiting highest S/R ratio are the best candidates for phytoextraction. The genotypes respond in a direction of accumulating a good amount of heavy metal from the sink and the interesting among these genotypes is that accumulation of metal does not affect much growth and yield. However, number of pods per plant reduced in heavy metal exposure but the Ni metal has got positive effects in number of seeds per pod. Thus our study indicates that this heavy metal has positive effects on the growth of mustard genotypes even at moderately higher concentrations

Conclusions

The results presented show the ability of *Brassica juncea* genotypes for Cd and Ni uptake, accumulation and tolerance. Our results showed that there is significant difference in root length, shoot length, fresh weight and dry weight between Cd and Ni treatments. *Brassica juncea* offers better accumulation potential for Cd compared to Ni.

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References

Blaylock, M.J., Huang, J. W. (2000). Phytoextraction of metals, In: Raskin and B.D. Ensley (Ed.) Phytoremediation of toxic metals: using plants to clean up the environment, John Wiley and Sons, Inc, Toronto, Canada, p-303.

Chaney, R.L., Angle, J. S., McIntosh, M. S., Reeves, R. D., Li, Y. M., Brewer, E.P., Chen. K.Y., Roseberg, R. J., Perner, H., Synkowski, E. C., Broadhurst, C. L., Wang, S., Baker, A. J. (2005). Using hyper accumulator plants to phytoextract soil Ni and Cd. Z Natuforsch **3-4**, 190-8.

Chang, P., Kim, J. Y., Kim, K.W. (2005). Concentrations of arsenic and heavy metals in vegetation at two abandoned mine tailings in South Korea, Environmental Geochemistry and Health **27**, 109-19.

Dang, H, Ye, Z.H., Wong, M. H. (2004). Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal contaminated sites in China. Environmental pollution **132**, 29-40.

Dan, T. V., Krishnaraj, S., Saxena, P.K. (2002). Cadmium and Nickel uptake and accumulation in scented Geranium (*Pelargonium sp.* Frensham). Water, Air & Soil Pollution **137,** 1-4.

Fayiga, A. O., Ma, L.Q. (2006). Using phosphate rock to immobilize metals in soil and increase arsenic uptake by hyperaccumulator *Pteris vittata*. Science of the

Total Environment **15,** 17-25.

Ingle, R. A., Mugford, S. T., Rees, J.D., Campbell, M. M., Smith, J.A. (2005). Constitutively high expression of the histidine biosynthetic pathway contributes to Nickel tolerance in hyperaccumulator plants. The plant cell **17**, 2089-2106

Kerkeb, L., Kramer, U. (2003). The role of free histidine in xylem loading in *Alyssum lesbiacum* and *Brassica juncea*. Plant physiology **131**, 716-724.

Mkandawire, M., Taubert, B., Dudel, E. G. (2004). Capacity of *Lemna gibba* L. (duckweed) for uranium and arsenic phytoremediation in mine tailing waters. International Journal Phytoremediation **6,** 347-62.

Ozturk, L., Eker, S., Ozkutlu, F. (2003). Effects of Cadmium on growth and concentrations of Cadmium, Ascorbic acid and Sulphydryl groups in durum wheat cultivars. Turkish Journal of Agriculture and Forestry **27**, 161-168.

Panwar, B. S., Ahmed, K. S., Mittal, S. B. (2002). Phytoremediation of nickel contaminated soils by *Brassica* species. Environment, Development and Sustainability **4**, 1-6

Quadir, S, Quareshi, M. I, Javed, S., Abdin, M. Z. 2004. Genotypic variation in phytoremediation potential of *Brassica juncea* cultivars exposed to Cd stress. Plant Science **167**, 1171-1181.

Rubio, M.I., Escrig, C., Cortina, M., Benet, F.J.L., Janz, D. (1994). Cadmium and Nickel accumulation in rice plants. Effects on mineral nutrition and possible interactions of abscisic and gibberllic acids. Plant growth regulation **14**, 151-157.

Schafer, H.J., Kerwer, A.H., Rausch, T. (1998). cDNA cloning and expression analysis of genes encoding GSH synthesis in roots of the heavy metal accumulator *Brassica juncea* L.: evidence for Cd induction of a putative mitochondrial gamma-glutamyl cycteine Synthetase isoform. Plant Molecular Biology **37**, 87-97.

Singh, R. P., H. B., Sharma, A., Rizvi, S. M. H., Jaiwal, P. K. (2001). Indian Mustard –a potential phytoremediator of heavy metal contaminated soil. Brassica **3**, 31-39.

Singh, K., Brar, J.S. (2002). Genotypic difference in effects of Cadmium on yield and nutrient composition in Brassica plants. Symposium No 42.Paper No-218. 17th World Congress of Soil Science in Bangkok, Thailand14-21 august.

Vassilev, A., Vangronsveld, J., Yordanov, I. (2002). Cadmium phytoextraction: present state, biological backgrounds and research needs. Bulgarian Journal of Plant Physiology **28**, 69-95.

Veselov, D., Kudoyarovu, G., Symonayam, M., Veselov, S. (2003). Effect of cadmium on ion uptake transpiration and cytokinin content in wheat seedlings. Bulgarian Journal of Plant Physiology. Special issue, 353-359

Wagner, G. J. (1993). Accumulation of cadmium in crop plants and its consequences to human health. Advances in Agronomy **51**, 173-212.

Wang, J. Q., Su, D.C. (2005). Distribution of cadmium in oil seed rape and Indian mustard grown in Cadmium contaminated soil. Journal of Environmental Science **17**, 572-75.

Zhu, Y.L., Pilon-Smits, E.A.H., Tarun, A.S., Weber, S.U., Jouanin, L., Terry, N. (1999). Cadmium Tolerance and Accumulation in Indian mustard is enhanced by Over expressing γ-Glutamylcysteine Synthetase. Plant physiology **121**, 1169–1177.

Zhu, Z.J., Sun, G.W., Fang, X.Z., Qian, Q.Q., Yang, X.E. (2004). Genotypic differences in effects of cadmium and elements in 14 cultivars of bai cai. Journal of Environmental Science and Health **39**, 675-87.

A Sino-Japan bilateral comparison study on evaluation and determination of rapeseed quality

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Abstract

A bilateral comparison study on evaluation and determination of rapeseed seed quality was carried out from 1999 to 2004 between China and Japan. The results showed that erucic acid, glucosinolates, oil, protein and chlorophyll content measured by Quality and Safety Inspection and Test Center for Oilseeds Products (QSITCOP), Agricultural Ministry of China, according to national standards of China and ISO standards were very close to the ones by Japan Oil stuff Inspectors' Corporation (NYKK) according to national standards of Japan and AOCS standards. The absolute differences between the results obtained by QSITCOP and NYKK were less than 0.41% for erucic acid, 5.82μmol/g for glucosinolates, 2.17% for oil content, 0.47% for protein content and 0.27% for chlorophyll content, respectively. Good reproducibility for erucic acid, glucosinolates, oil, protein and chlorophyll content determination with identical test material indicated that the analytical techniques and results of erucic acid, glucosinolates, oil, protein and chlorophyll content in rapeseed seeds measured by QSITCOP were internationally accepted. A platform was established for bilateral and multilateral authentication on analytical techniques of rapeseed seed quality. It is important not only to the improvement of rapeseed quality and analytical techniques, but also beneficial to international rapeseed trade.

Key words: Rapeseed; Quality; Bilateral Determination; Evaluation

Regards to the total output and production area of rapeseed, China where the consumption of rapeseed oil accounts for 35% of the total vegetable oil has been on the leading position in the world. Facing to the international competitive market and the impact from the entrance to WTO, establishment of the quality test methods and standards is very important not only to the improvement of rapeseed quality, but also to the international rapeseed export and import trade. Japanese people are very favor of rapeseed oil, and Japan where there is very little rapeseed production is a rapeseed importing country with very strict detecting technique and agro-product standard system. To improve analytical technique of rapeseed seed quality and establish the quality standards, a bilateral comparison study on evaluation and determination of erucic acid, glucosinolates, oil, protein and chlorophyll content in rapeseed seed was carried out from 1998 to 2004 between QSITCOP and NYKK supported by the Japan International Cooperation Agency (JICA).

1. Materials and method

1.1 Materials

Rapeseed produced in Hubei Province of China were selected as materials for comparison analysis.

In 1999-2000, erucic acid and glucosinolates contents in double low rapeseed varieties of zhongshuang 4, huashuang 2 and Qingyou14 collected by JICA program office, were analyzed by Quality Inspection and Test Center for Oilseeds Products, Agricultural Ministry of China with national standards of China and Japan Oil stuff Inspectors' Corporation with standards of JOCS.

In 2001, 2003 and 2004, samples from Shayang, Wuxue, angyang, Xiangyan and Qianjang where were the JICA observation bases in Hubei, China were used. Each sample was divided into 3 parts, and delivered to QSITCOP, NYKK and the JICA Project Office in Wuhan. Erucic acid, glucosinolates and oil content in 2001, glucosinolates, oil and protein content in 2003, erucic acid, glucosinolates, oil, protein and chlorophyll content in 2004 were analyzed and compared.

1.2 Method and apparatus

1.2.1 Erucic acid content analysis in rapeseed seed :national standards of China GB/T 17377－1998 and HP 5890IIGC were used by the QSITCOP in China. Basic method of 2.4.2-1996 edited by Japan oil chemists society and Hitachi 263－70 GC were adopted by NYKK in Japan.

1.2.2 Glucosinolates content analysis in rapeseed seed:ISO 9167-1 1992(E) and waters 5510/717/996 HPLC were used by the QSITCOP. AOCS Official Method AK1-92 and HP 1100 HPLC were applied by NYKK.

1.2.3 Oil content analysis in rapeseed seed:national standards of China GB/T 14488.1－1993 was used by the QSITCOP. Basic method for oil and fat 1.5-1996 was used by NYKK.

1.2.4 Protein content analysis in rapeseed seed:national standards of China GB/T 14489.2－1993 was used by QSITCOP. Basic method for oil and fat 1.7.2-1996 from JOCS was adopted by NYKK.

1.2.5 Chlorophyll content analysis in rapeseed seed:ISO10519:1997(E) was used by QSITCOP. Basic method for oil and fat 1.14-1996 from JOCS was applied by NYKK.

2. Results

2.1 Erucic acid content

The erucic acid content analysis was carried out in both laboratories with different instruments and methods. The results showed that absolute differences were from 0.00% to 0.41%,which was lower than the limit value of 3% within the acceptable standard errors (see table 1). It was indicated that erucic acid content measured by the QSITCOP in China according to national standard of China were very close to the ones by the NYKK in Japan according to national standard of Japan.

Table1 Erucic acid in rapeseed seed measured by QSITCOP and NYKK in China and Japan (%)

Year	Lab	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	QSITCOP	6.42	0.85	0.06		
1999	NYKK	6.50	0.60	0.10		
	Absolute differences	0.08	0.25	0.04		
	QSITCOP	2.02	2.36	0.28	0.30	
2001	NYKK	1.81	2.46	0.17	0.17	
	Absolute differences	0.21	0.10	0.11	0.13	
	QSITCOP	0.98	13.39	0.12	0.52	3.40
2004	NYKK	0.80	13.80	0.10	0.40	3.40
	Absolute differences	0.18	0.41	0.02	0.12	0.00

*In 1999, Sample was Zhongshuang No.4,Sample 2 was Huashuang No.2, Sample 3 was Qingyou No.14. In 2001 and 2004, Sample 1 from Shayang, Sample 2 from Wuxue, Sample 3 from Dangyang, Sample 4 from Xiangyan, Sample 5 from Qianjang

2.2 Glucosinolates content

The results of glucosinolates content in both laboratory with different instruments and methods based on 8.5% of water were shown in table 2. The absolute difference was 8.85μmol/g between the QSITCOP and NYKK, which implied there was a distinct difference because of different instruments, technicians and methods.

Since 2001, a Sino-Japan bilateral comparison study on evaluation and determination of rapeseed quality was supported by the JICA. The operation condition and instrument parameter were taken into account. The results showed that absolute differences were from 0.29μmol/g seed to 5.82μmol/g, which was lower than the limit of 8μmol/g, which was the acceptable standard errors (as shown in table 2). It was concluded that results of glucosinolates content measured by the QSITCOP in China according to ISO standard were very close to the ones by the NYKK in Japan according to AOCS standard.

Table 2 Glucosinolates content in rapeseed seed measured by QSITCOP in China and NYKK in Japan (μmol/g)

Year	Lab	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	QSITCOP	31.15	22.27	24.67		
1998	NYKK	40.00	27.00	23.00		
	Absolute differences	8.85	4.73	1.67		
	QSITCOP	24.05	20.09	11.59	12.11	
2001	NYKK	22.00	19.80	9.57	10.85	
	Absolute differences	2.05	0.29	1.84	1.26	
	QSITCOP	18.38	68.98	8.24		
2003	NYKK	19.50	74.80	6.90		
	Absolute differences	1.12	5.82	1.34		
	QSITCOP	24.93	42.43	15.05	16.25	28.31
2004	NYKK	21.70	29.4	18.1	19.2	23.8
	Absolute differences	3.16	3.03	3.05	3.05	4.51

*In 1998, Sample 1 was Zhongshuang No.4, Sample 2 was Huashuang No.2, Sample 3 was Qingyou No.14.In 2001,2003 and 2004,Sample 1 from Shayang, Sample 2 from Wuxue, Sample 3 from Dangyang, Sample 4 from Xiangyan,Sample 5 from Qianjang

Table 3 Oil content in rapeseed measured by QSITCOP and NYKK in China and Japan (%)

Year	Lab	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	QSITCOP	40.62	36.26	34.24	32.87	
2001	NYKK	41.99	37.86	35.17	35.04	
	Absolute differences	1.37	1.60	0.93	2.17	
	QSITCOP	34.05	35.58	33.52		
2003	NYKK	35.97	37.46	35.13		
	Absolute differences	1.92	1.88	1.61		
	QSITCOP	33.82	37.18	35.94	33.70	36.32
2004	NYKK	35.73	39.05	37.52	35.63	38.05
	Absolute differences	1.91	1.87	1.58	1.93	1.73

*In 2001,2003 and 2004,Sample 1 from Shayang, Sample 2 from Wuxue, Sample 3 from Dangyang, Sample 4 from Xiangyan,Sample 5 from Qianjang

2.3 Oil content

Oil content were measured by QSITCOP in China and NYKK in Japan in 2001,2003 and 2004 with the same rapeseed seed samples. The oil content in rapeseed seed was based on 8.5% of water (see table 3). The results showed that absolute differences were from 0.93% to 2.17%, which was higher than the maximal limit of 0.8%. It was found the reasons for the oil content differences between QSITCOP in China and NYKK in Japan, were from different grinding methods, extracting time and methods.

2.4 Protein content

In 2003 and 2004 the protein content in rapeseed seed was measured in both laboratories based on 8.5% of water (see table 4). The results showed that absolute differences was from 0.02％ to 0.47％, which was lower than the maximal limit value 1% of the acceptable standard errors of the methods.

Table 4 Protein content in rapeseed seed measured by QSITCOP and NYKK in China and Japan (%)

Year	Lab	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
2003	QSITCOP	22.47	23.04	25.43		
	NYKK	22.00	22.81	25.00		
	Absolute differences	0.47	0.23	0.43		
2004	QSITCOP	23.54	21.56	23.35	22.83	24.34
	NYKK	24.00	21.81	23.81	22.81	24.81
	Absolute differences	0.46	0.25	0.46	0.02	0.37

* Sample 1 from Shayang, Sample 2 from Wuxue, Sample 3 from Dangyang, Sample 4 from Xiangyan,Sample 5 from Qianjang

2.5 Chlorophyll content

Chlorophyll contents form the same rapeseed samples were measured by QSITCOP and NYKK in 2004. The chlorophyll content in rapeseed was calculated on the base of water 8.5% (see table 5). The results showed that absolute differences was from 0.05％ to 0.276％, which was within the acceptable standard errors of the methods.

Table 5 Chlorophyll content in rapeseed seed measured by QSITCOP and NYKK in China and Japan in 2004 (%)

Lab	Shayang	Wuxue	Dangyang	Xiangyan	Qianjang
QSITCOP	3.05	0.86	4.10	3.14	2.87
NYKK	3.10	0.80	3.90	3.40	2.60
Absolute differences	0.05	0.06	0.20	0.26	0.27

3 Discussion

More than two million tons of rapeseed seed were imported from Canada and China by Japan every year. There are very strict detecting technique standard and agro-product quality standard for the quality and safety control of rapeseed. Sino-Japan bilateral comparison study on evaluation and determination of rapeseed quality showed that the results of erucic acid, glucosinolates, oil, protein and chlorophyll content measured by the QSITCOP according to national standards of China and ISO standards were very close to the ones by Japan the NYKK according to national standards of Japan and AOCS standards although the different methods, condition and apparatus operated with different technicians. Good reproducibility of the results for erucic acid, glucosinolates, protein and chlorophyll content with identical test material in both laboratories indicated that the analytical techniques and results of erucic acid, glucosinolates, protein and chlorophyll content in rapeseed measured by the QSITCOP were internationally accepted. A platform was established for bilateral and multilateral authentication on analytical techniques of rapeseed seed quality.

Oil content measured by QSITCOP was lower than that from NYKK. The possible reasons for the differences may be the less grinding times without application of quartz during the grinding. It was put forward that the standard for oil content in rapeseed seed should be revised.

Content differences of glycoprotein and amino acids between self-compatible and self-incompatible lines in Yunjie (*Eruca Sativa* Mill.)

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Abstract

The glycoprotein and the components of amino acids in the stigmas of self-compatible and self-incompatible lines of Yunjie were extracted using hexane and ninhydrin and separated by SDS-PAGE. A protein band with MW of 50~55KDa was specific for self-compatible lines, but absent in the self-incompatible lines.[0] Seventeen types of amino acids (Gly, Met, Asp, Cys, Tyr, Lys, Thr, Val, Ser, Pro, Ile, Leu, Phe, Glu, Arg, Ala and His) were identified in the stigmas of Yunjie. The amount of amino acids in self-incompatible lines was higher than that in self-compatible lines in bud stage stigma, but in mature stigma, that was opposite. In self-compatible lines, Arg was 0.4758 mg and 2.7071 mg in both bud and mature stigma, respectively, which was comprised of about 54.1% and 25.8% of the total acids. In self-incompatible lines, Arg amount in both bud and mature stigma was 4.0733 mg and 0.1925 mg, respectively, and comprised of 60.8% and 17.5% of total acids. The amount of amino acid in bud stage stigma in self-incompatible lines was higher than that in self-compatible lines, but the amount of self-compatible was higher than self-incompatible lines in mature stigma, which suggested that the content of Arg might be related to self-compatible.

Key words: YunJie, glycoprotein, amino acid, self-incompatible

Introduction

Self-incompatibility formed in the evolution of plant (Goring1992; Nishio, 1992). It is a physiological reflection which aroused by reciprocity between farina and rumples cell of chapter. Self-incompatibility accelerates genetic habitability species and has important effects on polarization of species, especially on forepart evolution of angiosperm. It has been one of the hottest research areas in plant reproductive biology (Xue, 2002) for its importance in crop heterosis utilization.

Amino acids connect life activity and has special physiological function. It is one of the indispensable nourishment components in plants' body and is important in accelerating on plants growth (Schopfer, 2000). It is studied that self-compatible is connect closely with amino acid in chapter. The difference of kind and amount of amino acid can affect on protein synthesis, which will affect on all kinds of functions in cell (Wang, 2000). This paper studied the protein and amino acid of chapter of self-incompatible ESI1 and its self-compatible mutation ESC1.

Material and methods

This research chosen the chapter of Yunjie self-compatible (SC)and self-incompatible lines(SI) in former a blossom 2-3 days and after blossom1-2 days, The fresh material of chapter was conserved in refrigerator with -70°C for use. The chemistry reagents in this experimentation were as following: acryl amide, sodium laurel sulfate, glycogen, glycerol, phosphoric acid, Tris, acetone, acetic acid atrium et all. The apparatus in the experiment included amino acid automatism instrument, high speed refrigerated centrifuge, oven, electrophoresis chamber and so on.

0.2g material was quantified respectively and was placed in 2ml Eppendorf tube. and 1ml extracting solution(0.5MTris-HCl buffer, and pH=6.8) was added to the tube followed by distilling for 1h in ice bath and then centrifuge for10 minutes at 10000 rpm and 4°C. The supernatant fluid was transferred to another Eppendorf tube, -20°C chilled 10%TCA trichloroacetic acid was added according to 1:2.5(v / v) and the tube was shaken evenly at -20°C and then placed at -20°C for 2h for sedimentation protein followed by centrifuge for 15 min at 12000 rpm and 4°C. Then the supernatant luid was get rid of and the deposition was washed for 2-3 times with hypothermal acetone. The washed deposition was placed for 20 minutes to volatilize fully of acetone at-20°C. After that added protein lytic solution 200ul (62.5mmol/L Tris-HCl；2% sodium laurel sulfate；10% glycerol；5%β-sulfhedryl alcohol), and then boiled it for 2-3 minutes followed by SDS-PAGE electrophoresis to separate protein (Ji,1991).

Yunjie's fresh chapter of bud and maturation stage of SI and SC were chosen respectively. The material was placed into oven to dry and then grinded to powder. 100mg sample powder was measured precisely and put into 20ml test tube. Then added 3mol/L HCL to form 5ml solution, The tube was vacuum zed and sealed followed by being put in 110°C phosphoric acid bath for 24h and filtrated, The filtrate was transferred to capacitance bottle to suitable cubage (25ml), and then 1 ml was precisely extracted. Make it to be dry at 60°C by decompression, then 2ml ddH2O was added and braised to dry for two times. Finally, the residue was dissolved with 2 ml ddH2O, and the supernatant fluid was took to measure amino acid on the auto analyzer of amino acid (chromatogram condition: runoff: 0.22ml/min; the temperature of stove: 30°C; adopting the methods of grads washing and drafting) Measuration(Ai, 2005)

Results

1.Measurement sugar protein of chapiter in bud stage in SC and SI lines

The proteins of Yunjie in bud stage in self-compatible and self-incompatible lines were analyzed by the SDS-PAGE electrophoresis (Fig.1). There was a special band (about 50KD) in self-compatible (1,2 lines) between 66 KD and 45 KD on the gel electrophoresis map; but this band was absent in self-incompatible (3,4 lines). The electrophoresis band of self-compatible was stronger apparently than self-incompatible lines at about 18.0 KD. The electrophoresis bands of protein in bud stage chapter of self-compatible and self-incompatible lines were also analyzed statistically. The results showed that the self-compatible had 17 clearer strips. However, the self-incompatible had 16 strips. As analyzed above, a principium estimate that the difference strips of SC lines are connected with self- compatibility can be drawn.

2.The protein expression of autumn chapter in SC and SI lines

The results of the protein of Yunjie's autumn chapter in self-compatible and self-incompatible by the SDS-PAGE gel electrophoresis were shown in Fig.2. The results showed that the self-compatible had 19 clearer strips. However, the self-incompatible had 18 strips. The self-compatible lines were obviously brighter than self-incompatible lines at about 66 KD. A special strip (1, 2 lines) at about 50.5 KD was found in the self-compatible lines' strips. Comparing the gel electrophoresis maps of chapter in bud stage and autumn, the self-compatible lines had a strong different strip at about 66KD, but there is no change at about 50.0 KD. The expression quantity of every protein strip was different.

3. Amino acid of bud chapter and autumn of Yunjie SC

The content of amino acid of bud chipper and autumn were measured with amino acid automatism analysis. 17 amino acids in bud and autumn of Yunjie SC were found, including Gly, Met, Asp, Cys, Tyr, Lys, Thr, Val, Ser, Pro, Ile, Leu, Phe, Glu, Arg, Ala and His (table.1). The contents of Arg were the highest in both bud phase and autumn and arrived to 0.47580 and 2.70714 respectively. Among all these amino acids, the contents of Arg, Ala, Thr, Gly, Val, Pro, Leu, Met, His, Glu, Cys took on the trend of ascending from bud phase to autumn. Among them, Arg, Ala, Gly, Val, Leu, Met, His, Glu and Cys extended to significant difference level. The contents of Lys, Ser, Ile, Phe, Asp and Tyr took on the trend of descending. Among them, Lys, Ile, Phe, Asp and Tyr extended to significant difference level. The results indicated that the contents of most of amino acid in Yunjie SC took on the trend of going up from bud phase to autumn phase, and four kinds of amino acid were distinguished for their most increasing in content: Arg, Ala, Val, Cys, reaching 468.97%, 336.2%, 4014.27% and 104.27% respectively.

4. Amino acid of bud chapter and autumn of Yunjie SI

17 kinds of amino acids in the chapter of bud phase and autumn of Yunjie in SI lines were measured. Comparing all amino acids, it could be found that the content of Arg was the highest in bus phases (table.1), reached to 4.0733 and accounted for 60.8% of the whole amino acids. The content of Arg in autumn phase was 0.19252 and accounted for 17.5% of the whole amino acids. From the Fig.4,it could also be seen that the contents of some amino acids in bud phase, such as Arg, Lys, Ala, Gly, Val, Ser, Pro, Ile, His, Phe, Asp, Tyr ect, were far higher than that of autumn phase. Only the contents of Thr, Glu and Cys in autumn were higher than that of

bud phase. On the whole, the content of amino acid in chapter of SI takes on the descend trend.

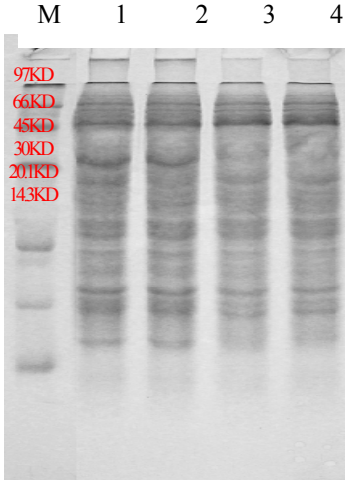

Fig.1 SDS-PAGE electrophoresis graphs of protein in bud stage stigma of self-compatible line and self- incompatible line in Yunjie Lines1,2 is self-compatible; Lines3,4 is self- incompatible

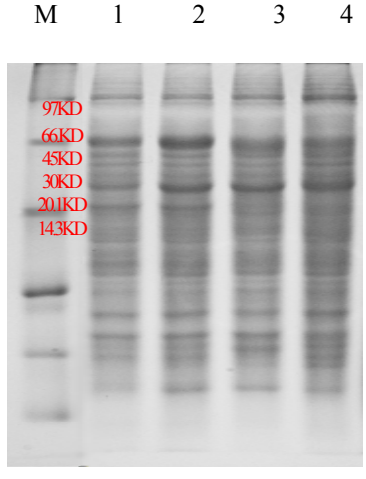

Fig.2 SDS-PAGE electrophoresis graphs of protein in mature stigma of self-compatible line and self-incompatible line in Yunjie. **Lines 1,2** is self-compatible**Lines 3,4** is self-incompatible

Discussion

The differences between SC lines and SI lines were obvious at about 50.5KD, 18.0KD and 66.0KD. The differences at

50.5 KD accorded with the former research conclusion: the main component of Brassica chapter rumbles cell is 45-55KD alkalescency sugar protein (chapter sugar protein is connected with SC property); Yunjie SC lines bud chapter map was different from SI lines; and expression quantity of protein was also different. In Yunjie SI lines, there was no difference in the map of bud phase and autumn. Based on the above analysis, the maps of chapter protein electrophoresis of Yunjie SI lines and SC lines were different significantly, which explained some protein expression were different significantly. Because special protein expression was connected closely with plants' hereditary information themselves, the special strips of SC lines may be relate to the property of its self-compatible, and the growth of plants anaphase chapter is based on the protein difference of bud phase in SC lines. From above results (Table.1), A conclusion can be drawn that In both SC lines and SI lines increasing and decreasing of Arg are both great in both bud phase and autumn, which is accordant with others research. It is thought that change of Arg is connected with SC property, Thus it can be seen that content of Arg has strong connections with self-compatibility.

Conclusions

The Yunjie chapter protein was measured and analyzed by technology of SDS-PAGE gel electrophoresis. From the Fig of electrophoresis maps, a special strips could be seen in Yunjie SC lines at about 50.5 KD in bud phase chapter. The strips of SC lines were much stronger than that of SI at about 18.0 KD and 15.5KD. SC lines bud chapter protein electrophoresis map had 17 strips, while SI lines had 16 strips; As shown in the mature chapter protein electrophoresis map(Fig 2), SI lines had 18 strips.

The amino acids of chapter in Yunjie SC and SI were measured and analyzed, the results indicated there were 17 kinds of amino acids identified. Arg distinguished itself for its highest content and highest change in SC lines and SI. In SC lines Content of Arg in bud phase arrive to 0.47580 and 2.70714 respectively and account for 25.8% and 54.1% of the whole content of amino acids respectively. In SI lines Content of Arg in bud phase and autumn arrive to 4.0733 and 0.19252 respectively, and account for 60.8% and 17.5% of the whole content of amino acids respectively.

Table.1Variance analysis of amino acid composition in stigma of self-compatible and self-incompatible line in Yunjie

	Amino acid	Material					
		Self-compatible line in Yunjie			Self-incompatible line in Yunjie		
		Bud stigma	Mature stigma	(±%)	Bud stigma	Mature stigma	(±%)
Amino acid composition (%)	Are	0.47580	2.70714**	468.97	4.07330**	0.19252	-95.27
	Lys	0.15480**	0.01422	-90.82	0.63247**	0.06809	-89.23
	Ala	0.05365	0.23404**	336.20	0.23400**	0.13235	-43.44
	Thr	0.01412	0.02688**	90.38	0.01315	0.03407*	159.19
	Gly	0.12327	0.28465**	130.91	0.02871**	0.01583	-44.87
	Val	0.01757	0.72287**	4014.27	0.61687**	0.33992	-44.89
	Ser	0.08548	0.07172	-16.09	0.05692**	0.02247	-60.52
	Pro	0.04734	0.04888	3.25	0.04889**	0.01378	-71.82
	Ile	0.08844**	0.02328	-73.68	0.34435**	0.01956	-94.32
	Leu	0.32237	0.37475**	16.25	0.09461	0.11437	20.89
	Met	0.01213	0.01829*	50.81	0.03397	0.01073	-68.43
	His	0.03087	0.08475**	174.53	0.05413*	0.03513	-35.09
	Phe	0.03402**	0.01260	-62.95	0.09526**	0.01508	-84.17
	Glu	0.10985	0.16279**	48.20	0.05443	0.09346*	71.706
	Asp	0.11489**	0.04366	-62.00	0.05001**	0.00579	-88.41
	Cys	0.07227	0.14763**	104.27	0.01640	0.04571**	178.69
	Tyr	0.10711**	0.02132	-80.094	0.24845**	0.00449	-98.19

Note: * mean significant difference at 0.05 level; ** mean significant difference at

References

Goring,D.R. et al., Identification of an S-locus glycoprotein allele introgressed from B.napus sap. rapiers to B. napus ssp. oleifera[J]. plant J, 1992,2:983-989.

Nishio, T.et al. Expression of S-loucs glycoprotein genes from Brassica oleracea and B.campestris in transgenic plants of self-compatible B.napus cv westar[J].explants report., 1992,5:101-109.

Xue Yongbiao,Cui Haiyang,Lai Zhao. Self-incompatible and its molecular biology bacia(2002) [M].The impregnation biology of angiosperm.

Schopfer CR, Nasrallah JB, Self-incompatibility: prospects for a novel putative peptide-signal molecule[J].Plant Physiol, 2000,124:935-940.

Wang Jiazheng, Fan Ming. The manual of protein technology[M].Sientific publishing company. the front page

Aug,2000.

Ji Yuming,YANG Gengrong,ZHong Rongren. The component of protein change in the calli of tobacco in adapt foe and maladjustment for salt. The Plant physiology, 1991, 17 (1) :56-62.

Ai ZHaohui,Guo Ling,He Men.The dissociated amino acid of was analyzed in the Overheat parabolas[J].Tropic medicine of China. 2005, 5 (7) :1440-1441.

Delimitation of local mustard (*Brassica juncea*) germplasm in Sri Lanka and improvement of their nutritive quality

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Abstract

Mustard (*Brassica juncea*) has been grown in the Indian subcontinent for hundreds of years as an oil seed crop, however, in Sri Lanka mustard is grown comparatively to a lesser extent. It is widely used as a condiment and oil is used in Ayurvedic medicines. There are *ca*. 60 mustard accessions available in Sri Lanka. However, the genetic diversity and the relationships among these mustard accessions are yet to be studied. The objective of this study was to assess the genetic divergence of local genotypes of *B. juncea* using numerical analyses of agro-morphological characters for delimitation and identification of genetically diverse and agronomically superior accessions. Thirty mustard accessions were selected and thirty five agronomic characters were measured. Data were analyzed using different multivariate statistical procedures; Cluster analyses (CA), Principle Component Analyses (PCA) and Discriminant Function Analyses (DFA). The results of the CA, PCA and DFA indicated that there is a difference in the grouping patterns of mustard accessions. Thus, there is a doubt that whether morphological characters are adequate in delimiting the mustard accessions. Therefore, the study suggests to include other sources of information such as biochemical evidence and molecular markers in characterization of mustard accessions. Local mustard consists of high amounts of unfavorable fatty acids (erucic acid) and low amounts of favorable fatty acids (oleic acid). High levels of erucic acid are associated with undesirable effects on cardiac muscles. Commercial canola (*B. napus*) varieties are nutritionally desirable for its monounsaturated property. Interspecific hybridization between six Australian commercial spring canola varieties and six local mustard varieties was successful in producing F_1 hybrid seeds. Regeneration of F_1 plants was achieved by embryo culture technique to overcome post-germination barriers using modified Lichter medium (Lichter 1982). Fatty acid analysis of F_1 seeds of all crosses with Gas Chromatography revealed a moderate amount of erucic acid (18-21%) compared to that of *B. juncea* (44-46%) and *B. napus* (0.2-0.5%). Oleic acid content in F_1 hybrids was improved (33-41%) compared to that of *B. juncea* (7-9%) and *B. napus* (43-57%). The study clearly indicated a higher possibility of transferring traits like a high level of oleic acid and a low level of erucic acid from canola to mustard and produce new mustard lines with an improved the fatty acid profile *via* interspecific hybridization.

Key words: mustard accessions, morphology, fatty acid profile, CA, PCA, DFA, interspecific hybridization, Sri Lanka

Introduction

On the basis of consumption and production of brassica oil seed crops are the third important after palm oil and soybean oil. It accounts for almost 14% of the edible vegetable oil supply of the world. Among many brassica species, *B.napus* L., *B.rapa* and *B.juncea* are considered as commercially important oil seed crops. In Sri Lanka mustard (*B.juncea*) is gown comparatively to a lesser extent and there are about sixty accessions (records of the Gene Bank of the Plant Genetic Resource Center-PGRC, Gannoruwa, Sri Lanka). These local accessions have undergone natural selection over a long period of time for desirable characters such as tolerances to drought, fungal and pest attacks.

Mustard oil contains high levels of nutritionally undesirable erucic acid. In Canada intensive breeding programs were developed to reduce the undesirable acids (erucic acid) during 1970's using the mutants of *B.napus*. Subsequently, released the first canola-quality cultivars created as a new, high-value oil and protein crop. Canola quality *B.napus* genotypes are often confined to temperate regions and they are not flowering in the tropics due to the thermo- and photosensitivity. Mustard has many advantages over canola which include more vigorous seedling growth, quicker ground covering ability, greater tolerance to heat and drought and enhanced resistance to the diseases blackleg and to pod-shattering (Burton 2004).

Recent studies have focused on the increased intake of saturated fatty acids (stearic and palmitic acids) and it's relative contribution to the increased serum cholesterol in the blood which increase the risk of coronary heart disease. The presence of polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acid are considered as desirable. The available canola oil is recognized as a superior dietary oil because it contains the lowest saturated fat level of any edible vegetable oil. Advantage of the use of high levels of oleic acid, linoleic acid, linolenic acid is to prevent cardiovascular diseases by inhibiting platelet formation and reducing cholesterol level. After carried out extensive nutritional studies in India, it has prooved that linoleic acid in mustard oil is highly beneficial for vegetarians and low income section of the society.

The present research is focused on characterizing and improving oil quality of local mustards by interspecific hybridization with commercial canola varieties.

Material and Methods

The accessions of *B. juncea* obtained from the PGRC, Gannoruwa, Sri Lanka, and six commercial spring canola varieties (cvs. 'Narendra', 'Hyola', 'Monty', 'Outback', 'Karoo', 'Oscar' obtained from Westerns Australia, were grown in the pots in the green house of the Institute of Fundamental Studies (IFS), Sri Lanka. Selected vegetative and reproductive characters were rerecorded at maturity of the plants.

Interspecific crosses: Flowers of female parent were emasculated and fresh pollen from the male parent was transferred to the stigma. A total of one hundred flowers were crossed for each parental combination. The pollinated flowers were tagged and bagged. Siliques were harvested 10-21 days after pollination and were surface sterilized and opened along the suture. The seeds were taken out and the seed coat removed to culture embryos. Embryos were transferred to water agar to test for germination. Non germinated embryos were transferred to hormone–free Murashige and Skoog (1962) medium (MS) and Lichter (1982) medium with 0.5 mgl^{-1} NAA, 0.5 mgl^{-1} BAP and 3% sucrose. Culture medium was solidified with 3 gl^{-1} agar. After adjusting pH to 5.7 the medium was autoclaved at 121^{o}C for 20 min at 15 psi. The embryos were transferred to Petri dishes and maintained under fluorescent light at 26±2 ^{o}C for a photoperiod of 16 h. The developing embryos were acclimatized before potting and transfer to thee greenhouse. The inflorescences were bagged at the flowering period to ensure self-pollination. Seeds were collected for fatty acid analysis.

Fatty acids were obtained by extracting 2 g of each seed sample (F_1 hybrids) in hexane on a Wristaction shaker overnight. The extracts were evaporated to dryness in a rotavapor, at 40^{o}C. Methanolic HCl was prepared by adding 5 ml of acetylchloride slowly to cooled dry 50 ml of methanol. The seed extract (100) mg was dissolved in this reagent and the mixture was heated at 50 ^{o}C overnight. This solution containing the methyl ester was analysed by Gas Chromatography on a DB-5 column.

Morphological characterization: The seeds of each accession were sown in plastic seed beds in a plant house at the Open University of Sri Lanka. A total of five seedlings of each accession were planted in black polythene bags with standard potting mixture. Subsequently, the seedlings were (3-4 leaf stage) transferred to plastic pots with a diameter of 13 cm. Each of these replicate was arranged in Randomized Complete Block Design (RCBD). Characterization of accessions was based on different morphological traits from seedling up to the harvest of the crop (Rabbani *et al*., 1988). The dataset was subjected to Cluster Analysis, Principle Component Analysis (PCA) and Discriminant Function Analysis (DFA) in order to classify the accessions and to trace the relationships among them. Further, these results were used to explore the importance of characters in classifying mustard accessions. Statistical analyses were carried out on SPSS/PC version 13.0 (SPSS/PC, 2004).

Results

The Fatty Acid Content (FAC) of the twelve accessions of *B. juncea* was dominated by the unsaturated erucic acid and the percentage was 41%. The mean monounsaturated oleic acid was 13.8%, and this amount is undesirable for edible purposes. Polyunsaturated linolenic acid was below 14% in *B.juncea* which is desired for a better shelf-life. In both species linoleic acids were at comparable levels.

Germination of the F_1 seeds was possible on a Listure medium. Seeds germinated on culture medium were successfully acclimatized and transferred to the green house. The Table 1 shows the difference in FAC between the parental species (*B. napus* and *B. juncea*) and their F_1 hybrids.

Table 1. Fatty acid content (%) of seeds of *Brassica juncea* (ac.7700), *B.napus* canola cultivars and their F_1 hybrids

Fatty acid	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Erucic (C22:1)
B.juncea	8	16	11	46
B.napus	47.2 ± 6.0	19.5 ± 1.4	11.0 ± 1.1	0.3 ± 0.2
F_1 hybrids	34.8 ± 3.9	17.1 ±1.3	10.3 ± 0.9	18.8 ± 1.4

The dendrogram (Figure 1) obtained from cluster analysis, showed five clusters of accessions at 60% phenon level. From the results of PCA, there was a particular grouping pattern within the accessions in which certain accessions were well-separated and other accessions overlapped considerably (Figure 2). The result of DFA indicated that there are three groups of accessions (Figure 3).

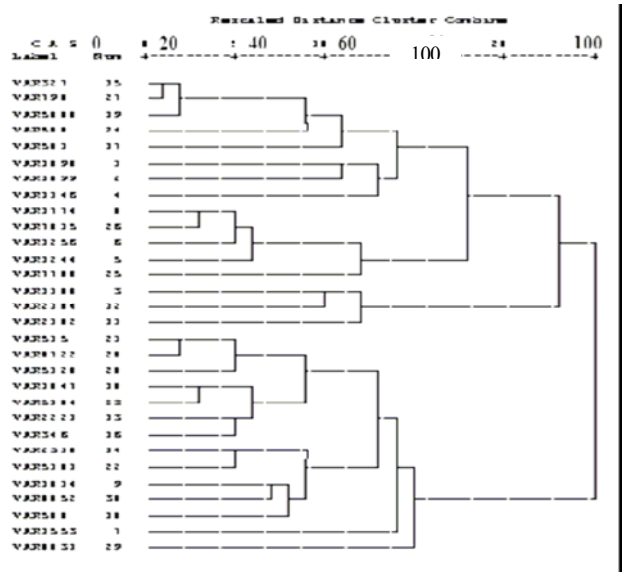

Figure 1 Dendrogram constructed from the cluster analysis

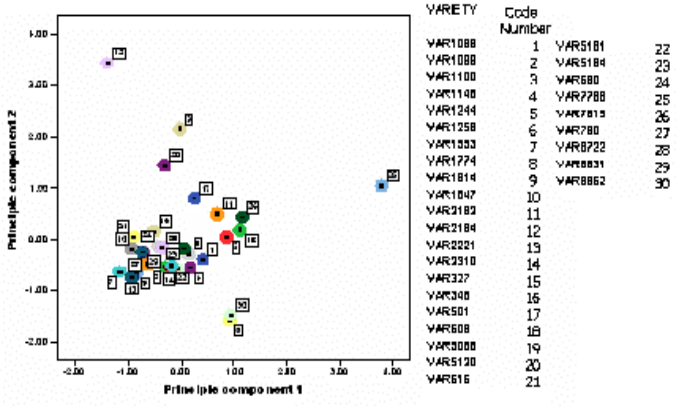

Figure 2 The "biplot" produced by plotting Principle Component axis 1 with Principle Component 2.

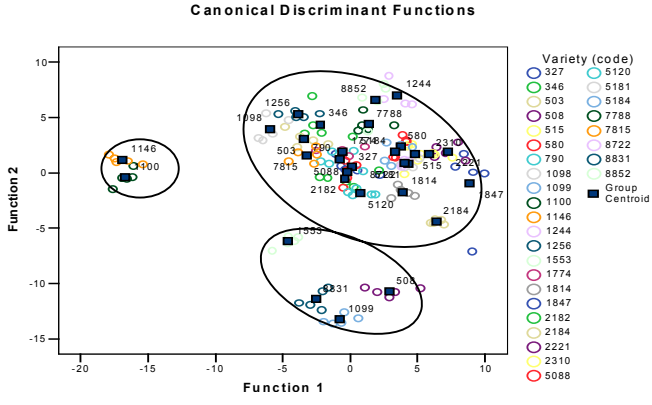

Figure 3 The scatter plot resulted from plotting Discriminant Function 1 with Discriminant Function 2.

Discussion

B.juncea grown in Sri Lanka contains nutritionally undesirable fatty acids and there is a need to improve the crop to use as a oil seed and it should be brought down to the levels of internationally and commercially accepted canola quality. By crossing *B.juncea* with *B.napus* we determine the feasibility of transferring the fatty acid profile to the canola quality. The inter-specific embryos were recovered by *in-vitro* embryo rescue, and raised them to maturity.

On cluster analysis the mustard accessions were fallen within five groups and this may be due to the similarities and relationships among accessions. However, PCA results were different from that of cluster analysis and this reflects the differences between the analytical methodologies. Since six out of thirty accessions were well-separated in PCA, these accessions could be maintained as separate accessions. Meanwhile, other accessions were broadly split into two groups with considerable overlapping. Therefore, the accessions in these two groups may be the same accessions with minor

morphological variation resulting from geographical variations and/or the difference in statistical analytical methods used in this study. The results of the DFA indicated that thirty accessions can be classified into three groups. Thus, grouping patterns of accessions were different under different statistical analytical methods.

Conclusion

Interspecific hybridization between *B.juncea* and *B.napus* produced F_1 hybrids with improved fatty acid profile. Since, there are inconsistencies in the results obtained from different statistical methods used in this study, morphological characters themselves are inadequate in characterizing mustard accessions and other sources of information such as isozymes, seed protein, seed fatty acids and molecular markers are of importance in characterization of mustard accessions grown in Sri Lanka.

References

Burton, S. J., Pymer, P. A., Salisbury, J. T., Kirk, O., and R. N. Oram. (2004). *Performance of Australian Canola quality Brassica juncea breeding lines.* (www.regional.org.au/au/gcirc/4/51.htm.).

Lichter, R. (1982). *Z. Pflanzenphysiol*. **105**: 427-434.

Murashige T,Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue culture. Physiologia Plantarum 15:437-497.

Plant Genetic Resources Catalogue. (1999). *Passport Information*. Plant Genetic Resources Center, Gannoruwa, Sri Lanka.

Rabbani, M. A., Iwabuchi A., Murakami Y., Suzuki, T. and K. Takayanagi. (1998). Phenotypic variation and the relationships among mustard(*Brassica juncea* L.) germplasm from Pakistan. *Euphytica* **101:** 357-366.

SPSS. (2004). SPSS/PC, Version 13.0. SPSS Inc., 444N, Michigan Avenue, Chicago, Illinois, USA.

Analysis of oil content in winter rapeseed (*Brassica rapa* L.)

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Abstract

The oil content of winter rapeseed was analyzed using Soxhlet extraction. The oil content of winter rapeseed was higher than spring rapeseed. The oil content of winter rapeseed was influenced by several factors such as altitude.

Key Words: winter rapeseed, spring rapeseed, oil content,variation

Introduction

Because the winter rape is self-compatible, drought-and cold resistant, long sowing time, early harvest, great productive potential and higher economic efficiency, the sowing area grows rapidly in Northwest, especially in Gansu province recent years. Oil content is the main concerned problem. We determined the oil content of *Winter Rape* varieties from different areas and expected to provide some scientific basis for the *Winter Rape* economic efficiency analysis and the variety breeding.

Materials and Method

The winter rape varieties of WYW-1, DQW-1, 9889, 9852, 02C za 9, Yanyou 2, MXW-1 and Tianyou 2 were collected from Wuwei, Zhangye and Jiuquan. The spring rape varities are mainly collected from Wuwei. Spring Rape sows in March, the Winter Rape sows in August.

Soxhlet extraction was used to determine the oil content. 2.5 grams of clean rape seed was weighed for each variety and dred at 80°C for 2 hours. The seeds were then put in a water extractor, ground into fine powder in mortar and packed with dry filters paper and dry at 105°C in the drying oven for 2 hours. Then the powder was taken out and weighed (A). Fill about 1 gram rape powder into a filter paper, and dry at 105°C in a oven for 3 hours, then take out and weigh (B). Fill the packages into the Soxhlet fat extractor and pour down ether till the ether completely soaks packages. After immersion in distilled water for at least 16h, heat up in water bath to auses the ether backflow. Control the backflow times in 8 times an hours, and generally extracts 6 to 8 hours. After extraction, takes out the package in to a ventilate place to let the ether volatize. Put the package in the drying oven at 105°C for 2 hours. Cool in the water extractor and weigh (C), the oil content (%) is calculated using equation (A-B)/(B-C).

Results and Analysis

Table 1 Oil contents of the winter and spring rape varieties

Varieties	number	Cultivars	Source	Oil content (%)	Average oil content (%)
Winter Rape	1	MXW-1	Wuwei	43.90	41.57
	2	MXW-1	Jiuquan	41.48	
	3	MXW-1	Zhangye	41.53	
	4	WYW-1	Zhangye	42.04	
	5	DQW-1	Zhangye	40.81	
	6	9889	Zhangye	40.35	
	7	9852	Zhangye	39.56	
	8	02C za 9	Zhangye	41.86	
	9	Yanyou number 2	Zhangye	42.28	
	10	Tianyou number 2	Zhangye	41.93	
Spring Rape	1	Wuwei small oil bud	Wuwei	37.00	39.67
	2	Wuwei small oil bud	Jiuquan	39.7	
	3	Wuwei small oil bud	Wuwei	41.02	
	4	Wuwei small oil bud	Wuwei	40.05	
	5	Wuwei small oil bud	Wuwei	40.60	
	6	Wuwei small oil bud	Zhangye	40.21	

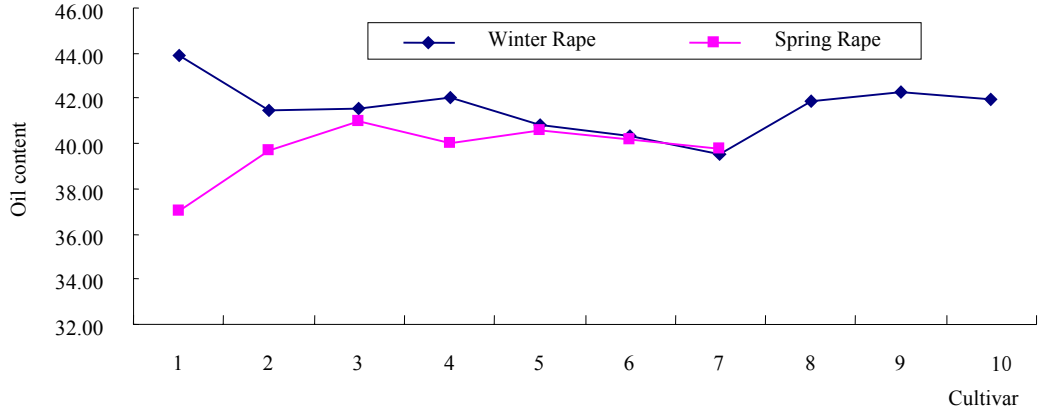

Figure 1. Oil contents of winter and spring rape cultivars

It can be seen from Table 1 that the average oil content of winter rape varieties' is higher than that of the spring rape (Figure 1). Oil contents of Winter Rapeseed and Spring Rapeseed from the same growing areas were compared and found that the Winter Rape varieties have higher oild content than the Spring Rapeseed varieties. The average oil content of Spring Rape grown in Wuwei is 37.00%, but is 43.90% in the Winter Rape varieties.

We compared the oil content of 8 Winter Rapeseeds sowing in Zhangye (Table 2). The Winter Rape cultivars average oil content is 41.05% (Table 2). The oil content ranges from 38.35% to 42.28%. Winter Rape varieties can be divided into two kinds according to oil content: oil content higher than 40.0% (WYW-1, DQW-1, 02C za 9, Yanyou 2, Tianyou 2r, MXW-1, 9889) and oil content lower 40.0% (9852).

Table 2 Oil contents of different Winter Rape cultivars

Winter Rape	Source	Oil content (%)
WYW-1	Zhangye	42.04
DQW-1	Zhangye	40.81
9889	Zhangye	40.35
9852	Zhangye	39.56
02C za 9	Zhangye	41.86
Yanyou number 2	Zhangye	42.28
Tianyou number 2	Zhangye	41.93
MXW-1	Zhangye	41.53

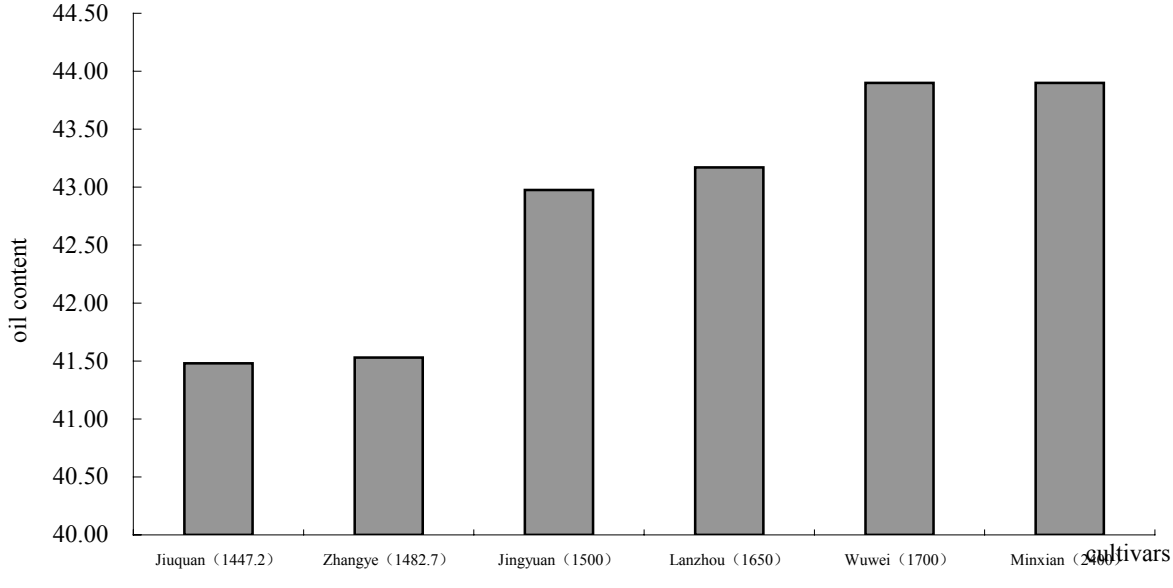

Figure 3 Influence of altitude on Winter Rape oil content

To test the relationship between oil content and the altitude, we grew the Winter Rapeseed variety MXW-1 at different

altitude (Figure 3). The oil content decreased as the altitude elevated.

Discussion

The results in this study indicated that the oil content of Winter Rapeseed was higher than Spring Rapeseed. The Winter Rape oil content is negatively influenced by altitude.

References

Wu Jie, Li Bao-Zhen 2004 Effects of different fertilizer levels on oil content of yellow-coated rapeseeds (Brassica napus L.) Chinese J Oil Crop Sciences. 26: 59-62.

Lemberkovics, E., Petri, G., Nguyen, H., Mathe, L., 1995. Relationships between essential oil and flavonoid biosynthesis in basil. Acta Hort. 426: 647–655.

Ram, M., Kumar, S., 1997 Yield improvement in the regenerated and trans-planted mint *Mentha* arvensis by r ecycling the organic wastes and manures. Biores. Technol. 59, 141–149.

Lewinsohn, E., Ziv-Raz,I., Dudai,N., Tadmor,Y., Lastochkin, E., Larkov,O., Chaimovitsh, D., Ravid,V., Pichersky, E., Shoham,Y. 2000 Biosynthesis of estragole and methyl-eugenol in basil (Ocimum basilicum Developmental and chemiotypic association of allylphenol O-methyl transferase activities. Plant Sci. 160: 27–35.

Ram, M., Ram, D., Roy, S.K., 2003 Influence of an organic mulching on fertilizer nitrogen use efficiency and herb and essential oil yieldsin geranium (Pelargonium graveolens). Biores. Technol. 87, 273–278.

Analysis of carotenoid in seed of several oil crops

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Abstract

Carotenoids are one of the most important members of plant pigments and have diverse functions such as antioxidant, preventing cardiovascular disease and cancer, anti-aging. Enhanced carotenoid content in seeds of oil crops improves not only the oil nutrition and health-care value, but also the oil stability. A fast, accurate, simple and convenient method was set up to extract and measure total carotenoid content in rape seeds. In addition, the carotenoid contents in seeds of six major oil crops including rapeseed, peanut, soybean, sesame, perilla and safflowers were analyzed, and compared with that of carrot, pumpkin, corn and orange. The results showed that in these six oil crops, only rapeseed and soybean demonstrated characteristic absorption peaks. In addiction, extensive variation in carotenoid content was detected among rapeseed and soybean varieties. The content of carotenoid in seeds of rapeseed is close to that of carrot. These results lay a good foundation for further screening of high carotenoid content rapeseed germplasm and the improvement of rapeseed and soybean varieties with high carotenoid content.

Key words: Carotenoids, Rapeseed,Mensuration, carotenoid content, oil crop, garden stuff

Introduction

Vegetable oil is an important component of human food. It supplies energy, nutrient, fatty acid source for human. Rapeseed, soybean, peanut, cotton seed, sunflower seed and flax are the main source of vegetable oil in China. The beneficial elements of edible oil have great function in preventing disease and enhancing health. For enhancing the content of beneficial health, it is stringent and practicable to intensify the nutrition, healthy care, and security of edible oil.

Carotenoids are fat-soluble and carotene-β is a precursor of vitamin A. They are able to quench single oxygen, remove harmful effects of the internal free ion-oxygen efficiently, protect the immune response system, and cure some serious diseases, such as cancer (Bartley et al., 1994). Carotenoids are not only in vegetable organ such as stem, leaf and root, but also in plant seeds. So it is possible to obtain carotenoids from edible oil. The content of carotenoid in edible oil can be improved by producing oil crops seeds with high content of carotenoids.

In this study, a fast, accurate, simple and convenient method was set up to measure the total carotenoids content in rape seeds by selecting the optimum conditions, such as solvents, time, the ration of rapeseeds on extraction process. Using the method, carotenoids content of six major oil crops including rapeseed, peanut, soybean, sesame, perilla and safflower analyzed and compared with that of carrot, pumpkin, corn and orange, providing important data base for crop breeding and correlation study of high carotenoids.

Material and methods

Material: Seeds of rapeseed, soybean, peanut, sesame, safflower, perilla came from Oil Crop Research Institute, carrot, pumpkin, corn and orange were bought from supermarket.

Methods: Seeds was pulverized in glass mortar, 0.3g was weighed accurately and filled in a triangular bottle with 9ml solution (petroleum ether:acetone, 1:1), soaked 6h in dark with shaking (100rpm). Then scan the solution within the range of 300-800nm and the absorption spectrum was obtained. Principle and method of mensuration about carotenoid content refer to GB/12291-1990 "the whole quantity mensuration of carotenoid in fruit and vegetable juice". Carotenoid content was calculated according to the following formula.

$$X(mg/100g) = \frac{A \cdot y(ml) \cdot 10^6}{A^{\%}_{1cm} \cdot 1000 \cdot g}$$

X—Carotenoid content. A—the highest absorbency value of 445nm Y—quantity of extracting solution
$A^{\%}_{1\,cm}$—average absorption coefficient 2500 of carotenoid molecule g—weight of sample

Result and Discussion

Measuring method of carotenoid in rapeseed

Scanning within 300-800 nm using spectrophotometer, a typical carotenoid spectrum absorbtion mode was obtained in rapeseed with the highest peak at 445 nm, two acromions at 421 nm and 473 nm (Fig. 1). The carotenoids in rapeseed can be dissolved in acetone, petroleum ether and chloroform. The best extraction condition is acetone and petroleum ether mixed by 1: 1 for 360 min, and the ratio of the seed and solution is 1∶30 (Gao, 2005)

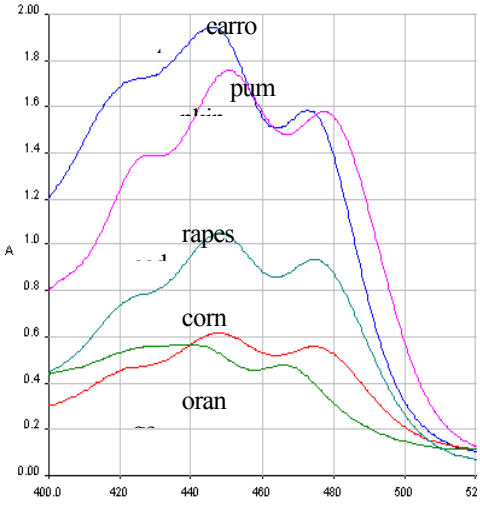

Fig. 1 UV-VIS spectra of carotenoid standard in fruit and vegetable

Carotenoid was previously reported in fruit and vegetable (Chug-Ahuja et al.,1993, Khachik,1992). There are typical absorption maximum of carotenoid in oilseed rape and four fruit and vegetable with the concentration of carrot and pumpkin solution diluted 5 times, the highest peak appears in 440-445nm (Fig 1), Which showed the typical absorption peak can be scanned by spectrophotometer in solution of carotenoid. Not only the content of carotenoid in fruit and vegetable but also of the oil crops can be analyzed by the method. The peak value, however, is different for different content and kind of carotenoids.

Rapeseed and soybean had absorption peaks of carotenoid with the highest peak at 443nm, two acromions at 419nm and 472nm. But the typical peak is absent in peanut, sesame, safflower, perilla, which nay contain less carotenoid (Fig. 2)

Content of carotenoid in six oil crops

The highest peak of carotenoid is in the range of 440±10nm by scan, so 443nm was used to calculate the content in the experiment with formula. The result showed the content has diversity for the different varieties in rapeseed and soybean. The average content of carotenoid was 1.426 mg/100g in *Brassica napus*, 3.342 mg/100g in *Brassica juncea*, 2.472 mg/100g in *Brassica campestris*, and 0.603 mg/100g in soybean that is lower than in rapeseed (Table 1). In addition, we found that the carotenoid content in *B.juncea* is higher than in *B.napus* and *B.campetris* by analyzing large number of varieties.

Table1 Carotenoid content in seed of different oil crops

Type	Name	Content of carotenoid (mg/100g)	Type	Name	Content of carotenoid (mg/100g)
B.napus	Ningyou 5	1.88	Soybean	xiangchun10	0.38
	Qianyou 1	0.83		zhongdou32	0.41
	Niujiaoyoucai	0.75		zhongdou29	0.80
	Huyou 2	1.36		01－45	0.67
B. campetris	qianyang259	2.31		Suzao 3	0.54
	Beishanyoucai	1.98		Zhe 3641	0.82
	huanggangbaiyoucai	2.17	Peanut	Zhonghua 12	ND+
	Xiezuo 1	2.49		Fenghua 4	ND+
	anqingziyoucai	2.66		99－1－2	ND+
	nanchangtianyoucai	3.06		Minhua 6	ND+
B. juncea	wensuheiyoucai	2.79		Jihua 4	ND+
	Shaosuhuangyoucai	3.16		984－12－4	ND+
	Tianjiazaiyoucai	3.72	Sesame	Zhongzhi 11	ND+
	Niuweihuang	1.85		Zhongzhi 13	ND+
	Yuxigaoke	5.19		Ezhi 1	ND+
Safflower	Honghua 3	ND+		Zhongzhi 10	ND+
perilla	0518 H37 baisu	ND+		Zhongzhi 14	ND+
	0512 H31 baisu	ND+		Zhongzhi 12	ND+
	0509 H28 zisu	ND+		Ezhi 2	ND+
	0520 H39 zisu	ND+		Zhongzhi 9	ND+

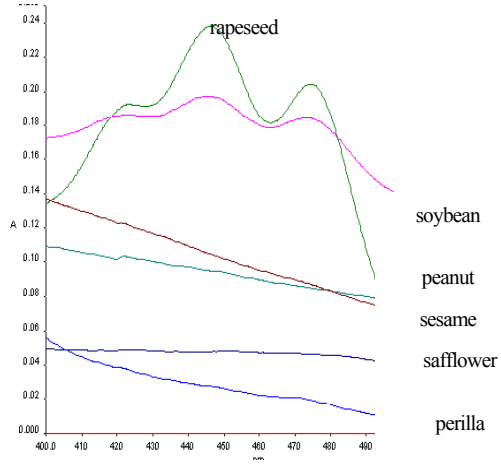

Fig. 2 UV-VIS spectra of carotenoid standard in six oil crops

Content of carotenoid in oilseed and four fresh fruit and vegetable

The carotenoid content in carrot is higher than the other three fruit and vegetable, and is close to that of some rapeseed varieties (Table 2). This showed that there exists abundant carotenoid in oil seeds. The content of carotenoid varies largely in rapeseed, which is propitious to select high content material for breeding.

Table 2 Carotenoid content of fresh fruit and vegetable and of rapeseed

Type	Content of carotenoid (mg/100g)
Carrot	6.13
Punpkin	3.24
Orange	4.19
Corn	0.91
rapeseed	0.75－5.19

Conclusion

The content of carotenoid can be analyzed by spectrophotometer through distillation with organic solvent in oil crops and fruit and vegetable. In six oil crops, only the rapeseed and soybean showed the absorption peak of carotenoid, and the content of carotenoid in some rapeseed varieties is close to that of carrot. So we can enhance content of carotenoid in edible oil through processing the seed of oil crops with high carotenoid content. Up to date, the biosynthesis approach of carotenoid has been illustrated with the development of biochemistry and molecular biology (Zhao et al., 2004). In addition, the key gene affecting and controlling the approach has been separated and identified (Fraser et al., 1994). So it is of high significance for enhancing carotenoid content of oil crops by regulation and controlling the process. Edible oil with high carotenoid content can improve the life quality and will bring significant profit for oil processing industry.

Acknowledgement

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References

Bartley G E, Sandmann P A, 1994,. *Annu Rev Plant Physiol Plant Mol Biol*, 45: 287-301.
Chug-Ahuja J K, Holden J M, et al. 1993. J Am Diet Assoc, 93:318-323
Khachik F M, Goli B, et al. 1992. Agric Food Chem: 56:128-135
GuiZ Gao& XiaoM Wu, 2005, Journal of plant genetic resources: 6 (4) :414－417。
WenE Zhao& YanJ Li, 2004. Acta Botanica boreal-occidentalia sinica: 24(5): 930-942.
Fraser P D, Truesdale M R, Bird C R, Schuch W, Bramley P M, 1994, *Plant Physiol*, 105: 405–413.

Rapeseed quality improvement according to survey in Yangtse valley of China

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Abstract

Yangtse valley has been the largest rapeseed production area in the world. Rapeseed quality and safety status in the valley influence the development of new cultivars and processing factors demanded by the end-user. This study was undertaken to compare quality improvement based on the data collected from the quality survey programs.

Harvest survey in Yangtse valley was carried out by Quality and safety Inspection and Test Center for Oilseeds Products of Agriculture Ministry of China (QITCOP) from 2002 to 2006. The analytical data presented were for all grades. All oil and glucosinolate content values were expressed on dry base in order to permit annual comparisons. About 300 samples were analyzed every year. Samples for the rapeseed harvest survey were collected from producers, crushing plants and grain handling officers across the Yangtse valley. These samples were cleaned to remove foreign matter prior to testing. Individual harvest sample are analyzed for oil, erucic acid and total glucosinolates using ISO reference method and national standard methods.

Harvest survey data for quality in the Yangtse valley oilseeds showed the yearly fluctuations in all quality parameters. Over the last 5-year period, the rapeseed quality of Chinese Yangtse valley has been gradually improved. Compared to 2002, the mean oil content of rapeseed in 2004 was as high as 42.7%, which was 1.7% higher than that in 2002. And the mean erucic acid was 3.14%, the mean glucosinolates in meal was 35.67μmol/g, which were both significantly lower than those in 2002.

Key words: Rapeseed, quality, Yangtse valley, China

Introduction

Rapeseed is one of the main oil crops in China.35% of total vegetable oil consumed in China is from rape oil. China is a big country for rapeseed plant area and rape oil consumption. The rapeseed production in China has been increased steadily since 1980s,especially during the last decade. The production acreage of repeseed is 6.25 million hectares, with 5.70~6.90 million hectares. It is three times more of the planting acreage in 1950s~1960s. And the total production has been increased 10 times. Yangtse valley has been the largest rapeseed production area in China and in the world. Rapeseed quality and safety status of the rapeseed in the valley influence the development of new cultivars and processing factors demanded by the end-user. Low erucic acid rapeseed oil was good for people nutrition and the low glucosinolate oil-extract cake was good feedstuff. Since early 1980s, the low erucic acid and low glucosinolate rapeseed has been planted in China, and rapeseed quality and safety status of the rapeseed has been experienced a tremendous increase in the last years.

This study was undertaken to compare quality improvement based on the data collected from the quality survey programs. Harvest survey in Yangtse valley was carried out by Quality and safety Inspection and Test Center for Oilseeds Products of Agriculture Ministry of China (QITCOP) from 2002 to 2006.

Material and methods

Data used in this study were derived from QITCOP harvest surveys for Yangtse valley monitoring programs conducted from 2002 to 2006. The analytical data presented are for all grades samples. All oil, glucosinolate content values are expressed on dry basis in order to permit annual comparisons. About 300 samples per year are used in the harvest survey. Samples for the rapeseed harvest survey are collected from farmers, producers, crushing plants and grain handling offices across Yantse valley. These samples are cleaned to remove foreign matter prior to testing. Individual harvest survey samples are analyzed for oil, erucic acid and total glucosinolates using ISO reference method and national standard methods.

The current oil content was determined by nuclear magnetic resonance (NMR) according to the International Organization for Standardization, reference number ISO 10565:1992(E) Oilseeds—Simultaneous determination of oil and moisture contents—Method using pulsed nuclear magnetic resonance spectroscopy. A Bruker NMS Minispec NMR Analyzer calibrated with appropriate oilseed samples extracted with petroleum ether was used. Results were reported as a percentage, calculated on a dry matter basis. Glucosinolate content was determined by International Organization for Standardization method reference number ISO 9167–1:1992(E), Rapeseed—Determination of glucosinolate content—Part 1: Method using high performance liquid chromatography. Results were calculated on a dry matter basis for oil-extract cake expressed as micromoles per gram (μmol/g). Erucic acid was determined by the International Organization for Standardization method reference number ISO 5508:1990 (E),Animal and vegetable fats and oils—Analysis by gas chromatography of methyl esters of fatty acids. A 15m by 0.32 mm column with a 0.25μm Supelcowax 10 coating is used,and esults were reported as a percentage.

Results and discussion

Harvest survey quality data for the Yangtse valley oilseeds showed the yearly fluctuations in all quality parameters (Fig 1). During the first 3 years, the mean oil content increased from 41.04% in 2002 to 42.64%.Then oil content fell to 41.03% in 2006. The mean erucic acid content decreased from 16.3% in 2002 to 3.14% in 2004, but increased to 12.87% in 2006. The mean glucosinolate content decreased from 64.87μmol/g in 2002 to 35.76μmol/g in 2004, then increased to 48.33μmol/g in 2006.

Over the last 5-year period, the rapeseed quality of Chinese Yangtse valley has been gradually improved. Compared to 2002, the mean oil content of rapeseed was as high as 42.7%, which was 1.7% higher than that in 2002. And the mean erucic acid was 3.14%, the mean glucosinolates in meal was 35.67μmol/g, which were both significantly lower than those in 2002.

Environmental factors such as heat, frost or drought may cause large annual fluctuations of glucosinolates, erucic acid and oil content. Due to double low breeding efforts, levels of seed glucosinolates and erucic acid in the oil have decreased and were well below rapeseed specifications. Changes in the erucic acid and glucosinolate contents over the past years may be a combination of environmental effects coupled with the shift in species from a mixture of *Brassica napus* and *B. rapa* to nearly exclusively *B. napus* types which traditionally had higher erucic acid and glucosinolates contents.

Table 1. Rapeseed quality data improved in Yangtse valley of China

Crop year	Oil content[1],%	Erucic acid,%	Glucosinolate content[2], μmol/g
2002	41.04	16.3	64.87
2003	41.54	4.94	47.15
2004	42.64	3.14	35.76
2005	40.63	7.73	48.93
2006	41.03	12.87	48.33
5 year mean	41.38	9.00	49.01

[1] dry matter basis
[2] dry matter basis for oil-extract cake

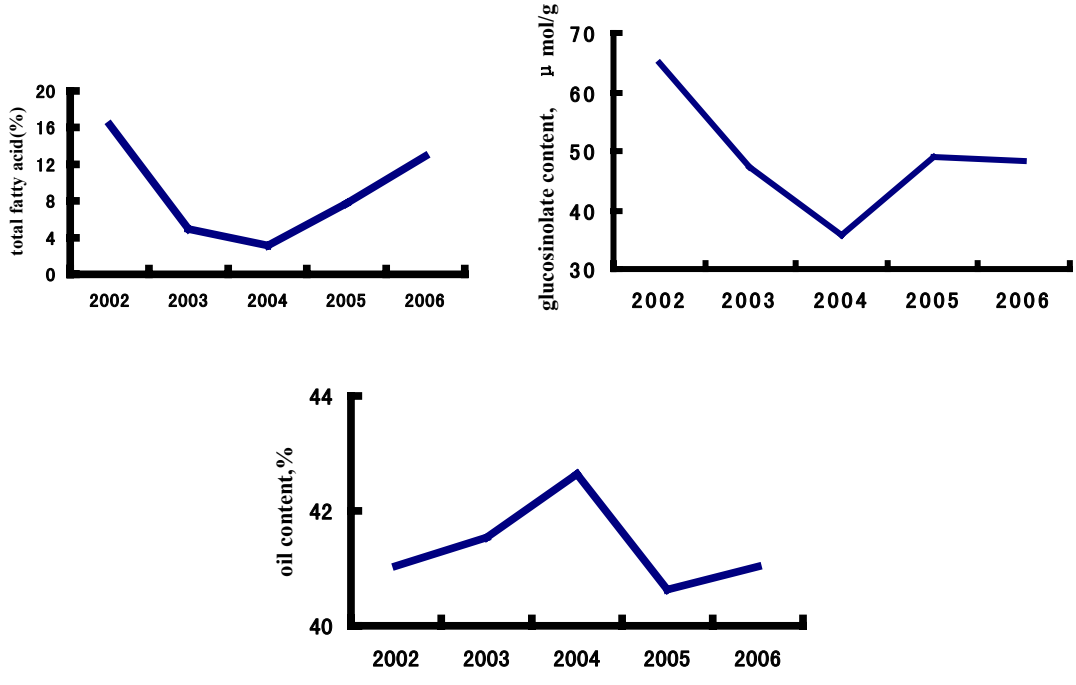

Fig.1 Comparison of apeseed quality parameters

Conclusions

Rapeseed harvest surveys for Yangtse valley monitoring programs have been a reliable predictor of the quality of all grades rapeseed quality in Yangtse valley. The harvest surveys provides an important scientific basis for the regionalization of rapeseed producing, the introducing varieties, the breeding of rapeseed varieties and the rapeseed scientific study in China. Based on the survey data in last five years, rapeseed quality in the Yangtse River valley has been significantly improved.

References

LI Pei-wu, YANG Mei, ZHANG Wen, CHEN Hong, XIE Li-hua, LI Guang-ming, DING Xiao-xia, WANG Xue-fang. (2004). Studies on quality of oilseed rape products and its improvement strategy in China. 84-88.

Fu Tingdong, Yang Guangsheng, Tu Jinxing, et al.(2001). The present and future of rapeseed production in China. Proceedings of International Symposium on Rapeseed Science. New York: Science Press, 3-5.

FU Ting dong, YANG Guang sheng, TU Jin xing, MA Chao zhi.(2003). The Present and Future of Rapeseed Production in China., China Oils and Fats.

WANG Han-zhong.(2005). Problem in the development of oilseed industry and it's countermeasure in China. Chinese journal of oil crop sciences. Vol 27, 100-105.

WANG Han-zhong.(2004). Technological Strategy to Improve the International Market Competition of Rapeseed Industry in China. Review of China Agricultural Science and Technology.

Rapeseed protein exhibit a poor digestibility but a very high metabolic utilization in humans

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Abstract

Rapeseed protein (*Brassica napus* L.) is only used in animal feed despite its high nutritional potential for human nutrition. We sought to assess the nutritional quality of rapeseed by measuring its real ileal digestibility and postprandial net protein utilization in humans fed ^{15}N-rapeseed protein. Volunteers equipped with an intestinal tube at the jejunal (n=5) or ileal level (n=7) ingested a meal containing 27.3 g ^{15}N-rapeseed protein and a total energy content of 2.93 MJ. Dietary N levels were quantified in intestinal fluid, urine and blood sampled at regular intervals during the postprandial period. The real ileal digestibility of rapeseed protein was 84.0 ± 8.8%. Dietary N at the ileal level was mostly in the form of undigested protein. Both cruciferin and napin fractions appeared to be resistant to proteolysis. Endogenous ileal N losses equalled 2.2 g/d. Aminoacidemia was not significantly increased by meal ingestion. The postprandial distribution of dietary N was 5.4 ± 1.8% in urinary urea and ammonia, 8.2 ± 3.4% in body urea and 7.7 ± 2.0% in plasma protein. The net postprandial protein utilization (NPPU) of rapeseed protein amounted to 70.5 ± 9.6% and the postprandial biological value was high: 83.8 ± 4.6%. Rapeseed protein has a low real ileal digestibility in humans compared to other plant proteins, but also exhibits a very low deamination rate. Thus postprandial nitrogen retention from rapeseed is excellent in humans, being as high as that of milk protein. We conclude that rapeseed protein has a high nutritional potential for human nutrition.

Key words: protein quality, rapeseed protein, ileal digestibility, biological value, humans

Introduction

Rapeseed (*Brassica napus*) contains a high proportion of protein (~ 20%) but, to our knowledge, there is no human consumption of rapeseed protein. Rapeseed proteins of quantitative importance are storage proteins: cruciferin (12S globulin), a globular protein rich in lysine and methionine, and napin (2S albumin), a soluble protein containing high levels of glutamine, proline and cysteine. The relative proportions of these proteins differ considerably between cultivars, with albumin levels ranging from 13% to 46% (*1*). In rat assays, indices for the nutritional quality of rapeseed protein were seen to be as high as those of animal protein and far higher than those of other legume or cereal sources (*2-4*). Studies of the nutritional quality of rapeseed proteins for livestock feed have shown that rapeseed protein is as efficient as soy protein in terms of animal growth and maintenance (*5*). In terms of its potential use for human nutrition, rapeseed protein is of particular interest because of its globally high content in indispensable amino acids (>400 mg/g protein) and particularly in sulfur amino acids (AAs) (40 to 49 mg/g protein) (*6, 7*). These levels are double the requirement for sulfur AA established following the last FAO/UNO/WHO consultation of experts in 2001 (*8*), and far higher than those usually found in plant, and especially, legume protein. However, protein quality does not only depend on AA composition but also on other factors related to the kinetics of AA delivery from these proteins. For instance, soluble milk proteins exhibit a high chemical score but because of the rapid supply of dietary AA from these proteins, their postprandial oxidation is enhanced when compared to proteins released at a slower rate (*9*). There has been no assessment to date of the digestibility or metabolic utilization of rapeseed protein which provides insights into its suitability and value for human consumption. In this context, our aim was to assess both the bioavailability and metabolic utilization of rapeseed protein *in vivo* in humans through the combined use of intestinal tubes and intrinsically and uniformly ^{15}N-labeled rapeseed protein.

Subjects and methods

Twelve subjects (6F, 6M) volunteered for the study. They were included after undergoing a thorough medical examination and routine blood tests. The subjects had a mean age of 25 years, weighed 71 ± 12 kg and their BMI was 23.4 ± 3.0 kg.m^{-2}. Body composition was determined from isotopic dilution after the oral administration of deuterium oxide (75 mg/kg body weight): total body water was 40.6 ± 5.8 L, fat-free mass 55.2 ± 7.9 kg and percentage fat mass was 23.1 ± 9.0%. All subjects received detailed information on the protocol and gave their written informed consent to

participation in the study. The protocol was approved by the Institutional Review Board for St-Germain-en-Laye Hospital, France.

^{15}N-labeled rapeseed protein was prepared at an experimental scale by growing at the Technical Centre for Oilseed Crops (CETIOM) winter rapeseed containing very low levels of glucosinolates (*Brassica napus* L., Goëland cultivar) in the presence of ^{15}N-ammonium nitrate. A rapeseed flour was produced from dehulled seeds by extraction with hexane to remove the oil (by CREOL, Pessac, France). The solvent was eliminated at a low temperature and under vacuum to protect protein functionality. A protein isolate was purified by solubilizing the rapeseed flour at pH 11 to eliminate insoluble polysaccharides, then adjusted to pH 7 and ultrafiltered at 20°C. The extraction and purification of rapeseed protein were carried out on a laboratory scale. In this cultivar, the globulin, napin and lipid transfer protein (LTP) fractions represent 36.8, 41, and 2.7% of total protein, respectively (*10*). The final N content of the rapeseed isolate was 14.9%, with ^{15}N enrichment of 1.16 atom percent (AP). The test meal consisted of 30 g of ^{15}N-labeled rapeseed protein isolate (312 mmol N or 27.3 g protein, N x 6.25), mixed with 96 g carbohydrate (75% as maltodextrin and 25% as sucrose), 23 g canola oil and water to reach a final volume of 500 mL. The total energy content of the experimental meal was 700 kcal.

The subjects were hospitalized for two days. On the first day, a 3-m PVC double-lumen tube was inserted via the nose under local anaesthesia and then swallowed so as to progress down the gastro-intestinal tract under the action of gravity and peristaltic contractions. Tube progression was verified under X-ray and the tip was halted in either the jejunum (n=5, mean tube length from the nose = 167 cm) or the ileum (n=7, mean tube length from the nose = 214 cm). The subjects were given meals at noon and 19h00 and then fasted overnight. On the second day, the protocol started at 10h00, when a saline solution containing 20 g/L polyethylene glycol 4000 (PEG-4000) was infused continuously through the first lumen of the tube. At t=0, the subjects ingested the experimental meal containing ^{15}N-rapeseed protein and 75 mg L-[1-^{13}C]-glycine given as a marker of the gastric emptying rate. Intestinal fluid, expired breath, blood and urine were sampled at regular intervals over a period of 8h.

Plasma and/or urine urea and ammonia were assayed using enzymatic methods. The PEG-4000 concentration in digesta samples was determined using a turbidimetric method. Amino acid concentrations in deproteinized serum samples were determined by HPLC after separation on cation exchange resin and post-column ninhydrin derivatization. For isotopic determinations, urea and ammonia were isolated from urine, using an Na/K form of the cation exchange resin. Serum separation of N fractions (protein N, free N and urea N) was performed. Protein N and non protein N in the ileal samples were fractionated by ethanol precipitation after hexane delipidation.The total N, non protein N and protein N contents of the digesta and serum protein fraction were determined using an elemental nitrogen analyzer. The ^{15}N/^{14}N isotope ratio was determined by isotope-ratio mass spectrometry in the digesta, urinary urea and ammonia, serum protein, free N and urea. The ^{13}C enrichment of CO_2 in expired breath was determined using GC-IRMS. The atom percent excess (APE) of the samples was calculated by subtracting the baseline value from the atom percent determined at each time point. Rapeseed isolate protein and ileal effluents were analyzed in polyacrylamide gels in denaturing (SDS) non-reducing conditions in order to determine the nature of undigested dietary protein.

Data are expressed as means ± SD. Changes over time of variables above the baseline value were tested using contrast analysis under a mixed model with time as a repeated factor (SAS 9.1, SAS Institute Inc., NC, USA). A value of $P < 0.05$ was considered as significant.

Results

Intestinal kinetics and real ileal digestibility of rapeseed protein

The flow of dietary N peaked 1h after the meal in the jejunum (14.9 ± 7.8 mmolN/30 min) and 30 min later in the ileum (8.7 ± 12.6 mmolN/30min). On average, over the 8-hour period, dietary N represented 42 ± 6% of total N in the jejunum and 37 ± 7% in the ileum. The cumulated recovery of dietary N was 29.9 ± 4.8 and 16.0 ± 8.8% of the ingested amount at the jejunal and ileal levels, respectively. The real ileal digestibility of rapeseed protein was 84.0 ± 8.8%. In subjects with the tube at the ileal site, analysis of the ileal N form (protein or non-protein) revealed constant levels of dietary non-protein throughout the postprandial period (0.1-0.3 mmolN/30 min) and high, variable amounts of dietary N in the form of protein, which accounted for more than 80% of the total ileal dietary N flux between 2 and 5h after the meal, reaching an average of 68% over the 8-h study period.As for ileal endogenous N flow, a high (mean: 79%), consistent proportion of endogenous N was made up of protein. Electrophoretic analyses were performed on the ileal contents in individual subjects and compared to the ileal dietary N flux. The profiles of the two individuals with the lowest degree of digestibility (65.2%) and highest degree of digestibility (90.5%) showed different profiles. In the first subject, peak levels of ileal dietary N (at 1.5 and 2h) in the ileum were associated with SDS-PAGE bands similar to those of the rapeseed protein (RP) isolate (at ~50 kDa and ~14 kDa, possibly due to the presence of undigested cruciferin (Cαβ) and napin (N1), respectively). These bands had a lower intensity in the subject with the highest degree of digestibility.

Kinetics of circulating glucose, dietary amino acids and dietary N-carrying protein

Plasma glucose levels rose after ingestion of the mixed meal, peaking at 8.4 ± 2.7 mmol/L at 1h. Plasma total amino acid concentrations ranged from 2975 ± 273 µmol/L at baseline to 3326 ± 761 µmol/L at 2 h (NS). There was no time effect regarding total, indispensable or dispensable AA levels after the meal.

Oxidation of the oral dose of ^{13}C-glycine and the incorporation of dietary N into the plasma amino acid N pool followed similar time-courses after the meal. ^{13}C excretion reached its maximum 3.5 h after the meal and represented 42 ± 3% of the

dose at the end of the 8-h period. The half-asymptotic excretion time, a proxy for the gastric emptying half-time, was 266 ± 24 min. Dietary N in plasma AA reached its maximum 3h after the meal (6.0 ± 2.0% of the pool). Dietary N incorporation into the plasma protein pool followed a sigmoid curve and reached 7.7 ± 2.0% of the N ingested, 8h postprandially.

Dietary nitrogen deamination, postprandial retention and postprandial biological values concerning rapeseed protein

The transfer of dietary N to body urea increased during the first 3h to reach a plateau with a final value of 6.7 ± 2.5% of ingested N in this pool. Low levels of ammonia were found in cumulative dietary N urinary excretion (0.23 ± 0.12% of ingested dose) but they increased regularly in urinary urea to reach 5.4 ± 1.8% of ingested N at 8h. Endogenous urea production remained steady (0.4 to 0.5 mmolN/kg body weight/2h) throughout the postprandial period and highly variable between subjects (Table 2). Urea production from dietary AA was at its maximum for the first two hours following the meal but almost insignificant during the last four hours. Total endogenous and dietary urea production amounted to 1.88 ± 1.41 and 0.47 ± 0.13 mmolN/kgBW over the entire postprandial period. The sum of ileal and deamination losses, representing the amount of dietary N not retained 8h after the meal, was 29.5 ± 9.6% of the meal content. As a result, the NPPU value was 70.5 ± 9.6%. The postprandial biological value, representing the retention of absorbed N, reached 83.8 ± 4.6%.

Discussion

This work constitutes the first determination of the nutritional value of rapeseed protein in humans. Using an intestinal tube to quantify ileal N flow rates and ^{15}N-labeled protein to specifically measure the metabolic fate of the dietary N absorbed, we showed that rapeseed proteins present a poor real ileal digestibility in humans (84%). This low bioavailability is compensated for by an excellent postprandial biological value (84%), higher than that of milk protein, indicating that the proportion of rapeseed derived-AA absorbed were only catabolized to a very limited extent. Taken together, these results indicate a postprandial retention of rapeseed protein of 70.5%, comparable to that of other plant proteins.

The real ileal digestibility (RID) of the rapeseed protein isolate reached 84%, a low value when compared to the RID of other plant proteins measured using the same methodology, which are all in the range of 89-91% (*11-15*), and to that of milk protein (95%) (*16, 17*) or egg protein using ^{13}C-protein (94%) (*18*). The RID of rapeseed was associated with broader variations between subjects than the aforementioned protein sources. This was due in particular to one subject with an extremely low RID of 65%.

Particular care was taken over preparation of the rapeseed protein isolate to avoid any drastic heat or alkaline treatment, thus the low RID measured could not be linked to any drastic technological treatment. In fact, our results agreed with pig data showing the lower apparent fecal digestibility of rapeseed protein than soy, and the lower true or real ileal digestibility (80-88%) of rapeseed protein than other plant proteins such as gluten, soy or pea (*7, 19-23*). Rapeseed protein has also demonstrated its poor digestibility in poultry (*24*), but not in rats (*3*). It is noteworthy that most of the animal studies used non-dehulled, rich in lignin rapeseed, which could partly explain the low level of digestibility observed. However, in dehulled rapeseed, proteins were still less digestible than soy protein (*25*). Thus, the low digestibility reported here probably resulted from the presence of hydrolysis-resistant sequences in the rapeseed proteins, the nature of which is unknown.

Our findings confirm the hypothesis that rapeseed contains protein fractions particularly resistant to hydrolysis. Based on the lower *in vitro* digestion of rapeseed protein vs. casein, it was hypothesized that pepsin may be less efficient on the highly compacted structure of rapeseed protein (*26*). Our comparison of the electrophoretic profiles of ileal samples and the corresponding ileal dietary N flux suggested that both the cruciferin fraction (α or β subunits, or their assembly into dimers) and napin were resistant to proteolysis. However, it was difficult to interpret some bands because rapeseed protein and endogenous protein, such as secretory IgG or pancreatic proteases have the same molecular weight. Our results therefore need to be confirmed using an accurate, quantitative method to determine the nature of indigestible dietary protein fractions in the ileum.

As a general rule, the globulin fraction of legume protein is seen to be more digestible than the albumin fraction (*14, 27-29*). Our results indicate that the difference is probably less marked for rapeseed protein, the 12S and 2S fractions of which may be resistant to digestion because of their very compact tertiary structure containing several disulfide bridges. An improvement in rapeseed protein digestibility may be achieved by heating to increase protein denaturation and its susceptibility to proteases, although this has not proved to be efficient in pigs (*25*).

Ingestion of the rapeseed protein isolate resulted in remarkably little deamination of dietary N, indicating that once absorbed, the catabolism of AA derived from the diet was minimal. This value is the lowest ever observed when studying the nutritional value of protein sources in humans: the deamination of dietary AA ranges from 16% (lupin) to 24% (wheat) (*12-15, 30*). The lack of plasma AA increase and the moderate incorporation of dietary N into both plasma AA and plasma protein after the rapeseed meal were consistent with the low deamination rate, as previously observed with milk protein compared to soy protein (*31*).

From our results, it does not appear that digestion kinetics of rapeseed protein could explain the high rate of dietary N utilization. It is more likely that the high rapeseed content in indispensable AA was responsible for its excellent postprandial biological value. Indeed, methionine and cysteine levels are as high as 19 and 20 mg/g of rapeseed protein, respectively, which is 80% higher than the limiting value for sulfur AA (met + cys = 22 mg/g protein) (*8*). This content is particularly high for legume proteins, which are usually limiting or sub-limiting sources of sulfur AA. Of particular interest is the high rapeseed cysteine content and the uncommon cys:met ratio of at least 1:1, comparable to that observed in egg protein. In growing rats, with high sulfur AA requirements, rapeseed is consistently particularly appropriate as a protein source (*2*). Rapeseed proteins

are thus promising, high-biological value proteins as a source of sulfur AA, which play a key-role for health, and notably cysteine as a precursor of glutathione (*32-34*).

Overall, the NPPU of rapeseed was 70.5%, a score comparable to the lower range of other legume proteins, and higher than that of wheat protein, a finding consistent with studies in the pig where rapeseed and soybean diets produce the same range of N retention (*5*) and exhibit no differences in terms of weight gain and lean carcass (*35*). Interestingly, a soybean diet produces lower fecal N losses but higher urinary N losses than a rapeseed diet, leading to the same overall N balance in pigs (*36*), a finding in close agreement with our observations in man. In rat assays, rapeseed protein generated some of the highest scores for plant proteins, being similar to beef and higher than casein (*2, 3*).

In conclusion, our study provides the first estimate of the nutritional quality of rapeseed protein in humans, achieved by determining the real ileal digestibility (84%) and NPPU (70%) of a rapeseed protein isolate given in a mixed meal to healthy subjects. Our findings show that this protein source could be of great interest to human nutrition. In particular, the high postprandial biological value of rapeseed protein was remarkable, presumably due to the high levels of indispensable AA and particularly sulfur AA, which exceeded those of all other legume proteins. An improvement in rapeseed digestibility, or the use of hydrolyzed or partially hydrolyzed rapeseed protein, are developments which might enhance the value of this protein source for human consumption.

References

B. Raab, H. Leman, K. D. Schwenke, H. Kozlowska, Nahrung 36, 239 (1992).
M. Friedman, J Agric Food Chem 44, 6 (1996).
G. Sarwar, World Rev Nutr Diet 54, 26 (1987).
L. U. Thompson, K. Boland, R. Chapkin, J. D. Jones, Nutr Rep Int 25, 621 (1982).
W. Grala, M. W. Verstegen, A. J. Jansman, J. Huisman, J. Wasilewko, J Anim Sci 76, 569 (1998).
J. M. Bell, M. O. Keith, Can J Anim Sci 71, 469 (1991).
W. Grala et al., Livest Prod Sci 48, 143 (1997).
P. Furst, P. Stehle, J Nutr 134, 1558S (Jun, 2004).
Y. Boirie et al., Proc Natl Acad Sci U S A 94, 14930 (1997).
C. Malabat, University of Nantes (2002).
N. Gausseres et al., Br J Nutr 76, 75 (1996).
C. Bos et al., Am J Clin Nutr 81, 87 (Jan, 2005).
F. Mariotti, S. Mahe, C. Luengo, R. Benamouzig, D. Tome, Am J Clin Nutr 72, 954 (2000).
F. Mariotti et al., J Nutr 131, 1706 (2001).
F. Mariotti, M. E. Pueyo, D. Tome, S. Mahe, Br J Nutr 87, 315 (2002).
C. Bos et al., Br J Nutr 81, 221 (1999).
C. Gaudichon et al., J Nutr 129, 890 (1999).
P. Evenepoel et al., Am J Physiol 277, G935 (1999).
J. M. Bell, M. O. Keith, Anim Feed Sci Tech 24, 253 (1989).
C. F. de Lange, W. B. Souffrant, W. C. Sauer, J Anim Sci 68, 409 (1990).
M. Imbeah, W. C. Sauer, R. Mosenthin, J Anim Sci 66, 1409 (1988).
W. C. Sauer, P. A. Thacker, Anim Feed Sci Tech 14, 183 (1986).
W. B. Souffrant, R. Lölher, G. Gebhardt, paper presented at the Physiologie digestive chez le porc, 1982.
Z. M. Larbier, A. M. Chagneau, M. Lessire, Anim Feed Sci Tech 35, 237 (1991).
W. Grala, M. W. Verstegen, A. J. Jansman, J. Huisman, P. van Leeusen, J Anim Sci 76, 557 (1998).
P. Valette, H. Malouin, T. Corring, L. Savoie, Br J Nutr 69, 359 (Mar, 1993).
M. Carbonaro, G. Grant, M. Cappelloni, A. Pusztai, J Agric Food Chem 48, 742 (Mar, 2000).
M. Le Gall, L. Quillien, J. Gueguen, H. Rogniaux, B. Seve, J Nutr 135, 1215 (May, 2005).
L. A. Rubio, G. Grant, P. Dewey, I. Bremner, A. Pusztai, J Nutr 124, 2204 (Nov, 1994).
C. Gaudichon et al., Gastroenterology 123, 50 (2002).
C. Bos et al., J Nutr 133, 1308 (2003).
A. K. Shoveller, B. Stoll, R. O. Ball, D. G. Burrin, J Nutr 135, 1609 (Jul, 2005).
G. Wu, Y. Z. Fang, S. Yang, J. R. Lupton, N. D. Turner, J Nutr 134, 489 (Mar, 2004).
N. K. Fukagawa, J Nutr 136, 1676S (Jun, 2006).
H. Siljander-Rasi, J. Valaja, T. Alaviuhkola, P. Rantamäki, T. Tupasela, Anim Feed Sci Tech 60, 1 (1996).
F. Schone, H. Ludke, A. Schneider, R. Zander, A. Hennig, Arch Anim Nutr 42, 11 (1992).

Inhibition of lipoxygenase activity by canola hull phenolic extracts

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Abstract

Crude phenolic (CP) extracts from Cyclone canola hulls were extracted into 30 to 80% (v/v) aqueous acetone and 30-80% (v/v) aqueous methanol and lyophilized. The 70% acetone and 80% methanol CP extracts were fractionated on a Sephadex LH-20 column into non-tannin and tannin fractions. The total content of phenolics in the prepared extracts ranged from 15 to 136 mg sinapic acid equivalents per gram of extract. Higher level of condensed tannins were detected in the acetone extracts than in the corresponding methanolic counterparts. Lipoxygenase (LOX) activity was assayed colorimetrically by measuring the conjugated dienes at 234 nm. The LOX inhibitory effect of CP extracts, IC_{50}, was expressed in mg extract required for a 50% inhibition of LOX activity in the reaction mixture. The 70% acetone and 80% methanol CP extracts displayed markedly stronger LOX inhibitory effects than any other CP extract investigated. The inhibitory effect of tannin fraction was over two times greater than that of corresponding CP extract. Statistically significant ($P \leqslant 0.05$) linear correlations were found between the IC_{50} values and total phenolic contents, the IC_{50} values and condensed tannin contents as well as the IC_{50} values and protein precipitating potential of CP extracts.

Key words: canola hulls, crude phenolic extracts, condensed tannins, lipoxygenase activity, inhibitory effects

Introduction

Lipoxygenase (LOX), when present in food, may contribute to the deterioration of food quality upon prolonged storage. LOX catalyzes the oxidation of polyunsaturated fatty acids to hydroxyperoxides (Eskin *et al*., 1977). These hydroxyperoxides are generally unstable and decompose to form a number of secondary oxidation products, such as carbonyls, that are responsible for off-flavors (Ridolfi *et al*., 2002). LOX inhibitory activity of phenols has been reported by a number of researchers. Examples of these are studies on LOX inhibitory activity of resveratrol (Pinto *et al*., 1999), anacardic acid (Ha and Kubo, 2005), octyl gallate (Ha *et al*. 2004), caffeic acid derivatives (Cho *et al*., 1991) and orange peel phenolics (Malterud and Rydland, 2000). The inhibitory effects of canola hull polyphenols on LOX activity is still not well documented.

Seeds of canola/rape contain 14-18% of hulls. Hulls may contain up 20% oil, 19.1% crude proteins [Nx6.25], 4.4% minerals and 48% of dietary fiber. Other constituents include simple sugars and oligosaccharides, polyphenolics, phytates and residual polar lipids (Naczk *et al*., 1994). Phenolic acids and their derivatives as well as soluble and insoluble condensed tannins are the predominant phenolic compounds found in canola and rapeseed. Canola and rapeseed hulls have been reported to contain up to 1000 mg of phenolic acids, from 89 to 1847 mg soluble condensed tannins and between 1913 and 6213 mg insoluble condensed tannins (Krygier *et al*., 1982; Naczk *et al*., 1994; Naczk *et al*., 2000). Therefore, the use of hulls, after dehulling, as a potential source of natural antioxidants may provide means for their utilization. The objective of this study was to investigate the inhibitory effects of phenolics extracted from Cyclone canola hulls on lipoxygenase activity.

Material and Methods

Cyclone canola hulls were prepared according to the procedure described by Sosulski and Zadernowski (1981). The hulls were extracted for 12 h using a Soxhlet apparatus and dried at room temperature. Cyclone canola hulls were extracted twice at room temperature into 30-80% (v/v) aqueous acetone or 30-80% aqueous methanol using a Waring Blender for 2 min at maximum speed. The extracts were combined, the solvent was removed under vacuum at <40 ^{o}C, and then the crude phenolic extracts were lyophilized.

The content of condensed tannins in the CP extracts was estimated by the modified vanillin assay (Price *et al*., 1978) and expressed as mg catechin equivalents/g extract. The protein precipitation capacity of CP extracts was measured using the dye-labeled protein assay of Asquith and Butler (1985) (2 mg dye-labeled BSA/mL) with modifications as described by Naczk *et al.* (1996).

The lipoxygenase (LOX) activity was assayed colorimetrically as described by Liu and Pan (2004). The reaction mixture consisted of 100 μL of 0.33 μL/mL arachidonic acid (suspended in 0.05M phosphate buffer pH 7.0 containing 0.4 μL/mL Tween-20), 200 μL of 1mg/mL of soybean lipoxygenase (dissolved in assay buffer), 100 μL of methanolic solution of CP extracts (0.2-1.2 mg/mL of CP extract) and 1.8 mL of assay buffer (0.05M phosphate buffer pH 7.0 containing 2 μM/mL of glutathione and 0.4 μL/mL of Tween-20). The reaction mixture was incubated at 20 ^{o}C for 6 min. The absorbance at 234 nm was read at 30 sec intervals. The inhibitory effect of phenolics on LOX activity was expressed in μg of CP extract/assay

required to reduce the LOX activity by 50%.

Statistical analysis of data was carried out using the SigmaStat v.3.0 (SSPS Science Inc., Chicago, IL, USA). Each extract, for the purpose of statistical analysis, was referred to as treatment. The statistical analysis of all treatments was perfomed using the ANOVA test. In addition, the t-test was employed among the treatments when a statistically significant difference ($P \leqslant 0.05$) was found using the ANOVA test. The results presented in tables are graphs are mean values of at least three experiments. Treatments followed by the same subscript letter in a tables are not significantly different ($P > 0.05$; t-test).

Results and Discussion

The crude extracts of phenolics from plant material are complex mixtures of phenolics with different molecular structures. Phenolic acids and condensed tannins are the predominant classes of phenolics found in seeds of canola and rape. Therefore, the Folin-Denis, vanillin and proanthocyanidin assays were selected for estimation of the content of phenolics in CP extracts. Table 1 summarizes the total phenols and condensed tannin contents in canola hull crude phenolic (CP) extracts. The total phenolics content in the CP extracts was between 15.1 and 103.8 mg sinapic acid equivalents/g of extract, while the content of condensed tannin (as determined by the vanillin assay) ranged from 3.5 to mg/g extract (for 30% v/v MeOH) to 238.0 mg/g extract (for 80% v/v acetone). The acetone-water solvent systems were more efficient for extraction of phenolics than corresponding methanol-water solvent systems. Of these, 70 and 80% (v/v) acetone was the most effective solvent for extraction of canola hull phenolics.

Table 1 Total Phenolics and Condensed Tannins Content in Canola Hull Extracts

Solvent System [%v/v]	Methanol			Acetone		
	TP	VAN	PROANTH	TP	VAN	PROANTH
30	15.1±0.1	3.5±0.6	16.8±1.0	21.2±0.2	10.8±0.1	32.9±2.0
50	23.9±0.2	4.4±0.2	25.0±0.6	41.5±0.3	40.1±1.4	95.3±2.0
70	36.5±0.6	6.9±0.2	41.4±0.9	94.3±3.0	127.0±2.5	224.4±9.2
80	40.4±0.2	6.0±0.2	45.6±1.6	103.8±2.7	238.0±5.3	296.9±8.6

TP- Total Phenolics by the Folin-Denis assay; units mg sinapic acid/gram of extract; VAN - condensed tannins by the vanillin assay; units are in mg canola tannins/ gram of extracts; PROANTH –condensed tannins by the proantho-cyanidin assay; units are in absorbance units/ gram of extract.

A number of methods have been developed for estimation of protein-precipitating potential (PPP) of plant-based phenolic extracts. Of these the dye-labeled protein assay of Asquith and Butler (1985) was selected for determination of PPP of CP extracts. The assay measures the amount of protein precipitated by phenolics. The PPP of CP extracts was expressed as a slope value of line depicting the amount of protein-phenolic complex precipitated as a function of the amount of extract added to the reaction mixture

(Naczk *et al*., 2001). Table 2 summarizes the PPP of CP extracts and inhibitory effect of CP extract on the LOX activity. Acetone–based CP extract displayed stronger affinity for proteins than those of corresponding MeOH extracts. According to Porter and Woodruffe (1984) the ability of phenolics to precipitate proteins depends on their degree of polymerization. Furthermore, the acetone-based CP extracts also had a stronger inhibitory effect on LOX activity than those of corresponding MeOH extracts. This may be brought about by the differences in total phenolics and condensed tannins contents, as well as by the diversity in molecular structures of phenolics present in CP extracts.

Table 2. Protein precipitating potentials and inhibitory effects of canola hulls phenolic extracts on LOX activity

Solvent System [%v/v]	Methanol		Acetone	
	DPL	IC_{50}	DPL	IC_{50}
30	0.7	2867	-	2352
50	2.4	2224	3.4	1526
70	3.0	1681	9.5	838
80	4.3	1267	9.7	735

DPL -the dye-labeled protein assay; units: %precipitated proteins/g extract

IC_{50} - μg of extract/assay required to reduce the LOX activity by 50%.

Statistically significant correlations (power function) existed between the LOX activity and the total phenolics and condensed tannins content as determined by the proanthocyanidin and dye-labeled protein assays (Figure 1). These correlations suggest that condensed tannins contribute significantly to the inhibitory effects of CP extracts on the LOX activity.

References

Asquith, T. N.; Butler, L.G. 1985. *J. Chem. Ecol.* 11: 1535.

Cho, H.; Ueda, M.; Tamaoka, M.; Hamaguchi, M.; Aisaka, K.; Kiso, Y.; Inoue, T.; Ogino, R.; Tatsuoka, T.; Ishihara, T.; Noguchi, T.; Morita, I.; Murota, S-I.. 1991. *J. Med. Chem.* 34: 1505.

Eskin, N.A.M.; Grossman, S.; Pinsky, S. 1977. *Crit. Rev. Food Sci. Nutr.* 9: 1.

Ha, T.J.; Kubo, I. 2005. *J. Agric. Food Chem.* 53: 4350.
Ha, T.J.; Nehei, K.; Kubo, I. 2004.*J. Agric. Food Chem.* 52: 3177.
Krygier, K.; Sosulski, F.; Hogge, L. 1982. *J. Agric. Food Chem.* 30: 334.
Liu, Y.J.; Pan, B.S. *J. Agric. Food Chem.* 52: 4860.
Malterud, K.E.; Rydland, K.M. 2000. *J. Agric. Food Chem*. 48: 557.
Naczk, M.; Nichols, T., Pink, D., Sosulski, F. 1994. *J. Agric. Food Chem.* 42:2196.
Naczk, M., Oickle, D., Pink, D., and Shahidi, F. 1996. *J. Agric. Food Chem.* 44: 2144.
Naczk, M.; Amarowicz, R.; Pink, D. Shahidi, F. 2000.*J. Agric. Food Chem.* 48: 1758
Naczk, M.; Amarowicz, R.; Zadernowski, R.; Shahidi, F. 2001. *J. Am. Oil Chem. Soc.* 78: 1173.
Price, M.L.; van Scoyoc, S.; Butler, L.G. 1978.*J. Agric.Food Chem.* 26: 1214.
Pinto, M.C.; Garcia- Barrado, J.A.; Macias, P. 1999. *J. Agric. Food Chem.* 47: 4842.
Porter, L.J.; Woodruffe, J. 1984. *Phytochemistry* 25:1255.
Price, M.L., van Scoyoc, S., Butler, L.G. 1978. *J. Agric. Food Chem.* 26: 1214.
Ridolfi, M.; Terenziani, S.; Patumi, M.; Fontanazza, G. 2002. *J. Agric. Food Chem.* 50: 835.
Sosulski, F.W.; Zadernowski, R. 1981. *J. Am. Oil Chem. Soc.* 58: 96.

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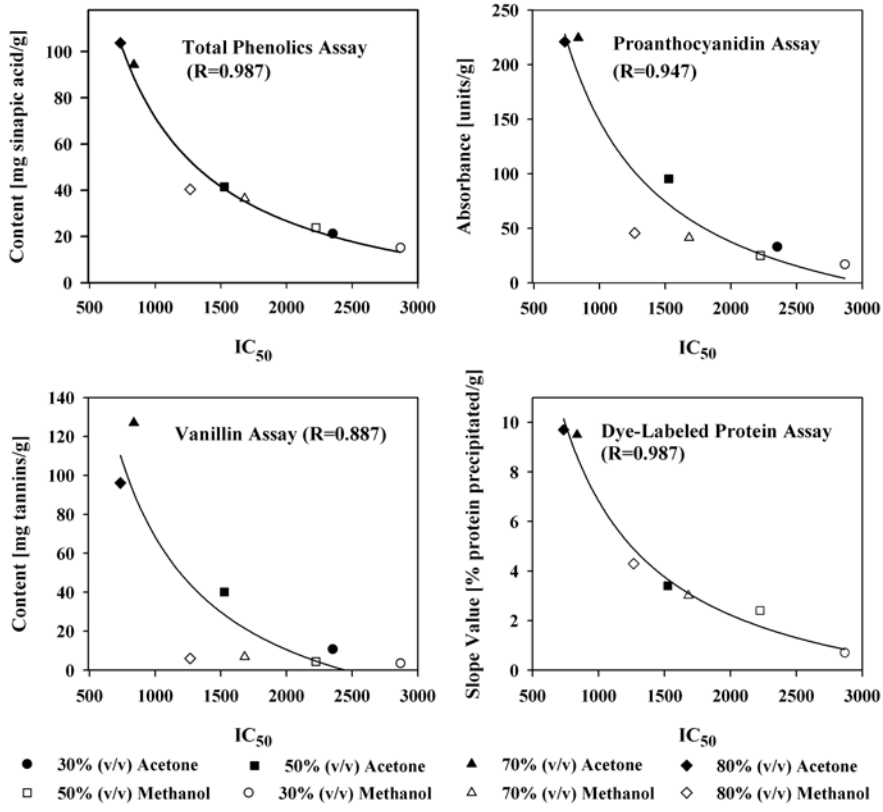

Fig. 1. Effects of total phenolics, and condensed tannins as determined by the vanillin, proanthocyanidin and dye-labeled protein assays on the LOX activity.

Contribution to understand the fluctuation of linolenic acid profile in winter oilseed rape grown in France

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Abstract

According to the increase of rapeseed oil for human consumption in France, the linolenic acid content (C18:3 n-3) of 9% is required. The authors brink their contribution to understand the fluctuation of this criteria. Genetic effects were underlined and could be used for recommended list of varieties. Locations induced also high fluctuations. On this point, in relation with the accumulation of the fatty acids in the seed during an early sensitive window, the authors demonstrated that the most important criteria was the cumulated minimum temperature during a sensitive period of 60 days (flowering to flowering + 60 days). The model identified that to reach the higher value (i.e. > 9% of C18:3), the cumulated minimum temperature needs to be below 450 °c base 0). The model was established during two years (2004 and 2005), and tested in 2006. The application of such a result could be to predict before harvest the average value for the linolenic acid content and to advise for dedicated the seeds from one area to special use for labelled oil with a linolenic acid certificated over 9%.

Key words : oil, linolenic acid, accumulation, prediction

Introduction

Following the studies conducted on oilseed rape, there is now evidence that the rape oil get really an interest for human consumption and mainly in prevention of heart attacks and in reducing cholesterol levels in blood. This quality is mainly related to fatty acids profiles and especially in the linolenic content (C18:3, omega 3) of the oil.

According to a survey conduct in France, we identified a fluctuation of these criteria. The data published in the *codex alimentarius* indicate an average value of 9%. Our results bring a contribution to understand the mains factors involved and to help the crushers to select varieties and/or locations to collected seed according to the requirement of the market. For example, some oils crushers developed a market for rape oil certificated at 9% of C18:3. Previous results obtained by Izquierdo and al. (2002) on sunflower indicated that the most important criteria to explain the fluctuation of fatty acid in the oil was the night temperature during an early sensitive period taking place 10 days after anthesis (DAA) to 30 DAA. Using this threshold, Merrien and al., (2005) put evidence that it was possible to explain the variation of fatty acid composition in sunflower oil according to different location in France. A strong correlation was obtained between the minimum temperature during this period and the fatty acid content in the oil. The greatest were the values, the highest was the oleic content. The model was valid for conventional type as for oleic type.

Tremolières (1978), Deng and Scarth (1998) demonstrated also the strong effect of temperature on fatty acids equilibrium. Champolivier and Merrien, (1993) show that the most active period for fatty acid accumulation in the rape seed take place during 60 days after anthesis 60days.

Materials and methods

We collected during 3 years (i.e : 2004, 2005 and 2006) samples from fields plot experiment through 3 locations in France : the East (Nancy), the Centre (Bourges) and the Atlantic border (Surgères). Those samples included each year a panel of the most important varieties grown in France.

For each sample (varieties/years/locations, we check the growth stages (mainly anthesis). The climatic datas was collected from the nearest climatic stations. According that night values was not available, we set up the hypothesis that the minimum temperature was the night one. The cumulative values, day by day, was calculated during the sensitive period (during 60 days after anthesis - DAA).

The fatty acids profiles were check by gas chromatography following the ISO method (NF EN ISO 5508).

Results

Interrelation between fatty acid in the oil

From our data set, the fluctuation of oleic content range from 59 to 68% of the total fatty acid content in the oil. As far as linolenic fraction is concerned, the fluctuations range from 11 down to 6 %. The figure 1 indicated the inverse relation between the main fatty acid composition in 2004: as the oleic fraction increase, the linoleic (C18:2) and the linolenic (C18:3) fraction decrease. The same figure was obtained for 2005 and 2006.

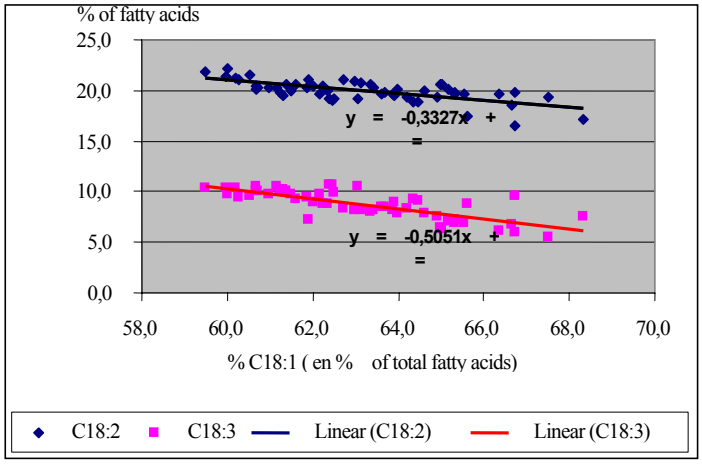

Figure 1: Relations between the3 main fatty acid content in the rapeseed oil collected from different location in 2004.

Variations according to genotypes

The table 1 summarized the results obtained during the 3 years for the most important varieties in the data set. We identified for example that the profile for variety *Pollen* was lower for C18:3 compared to the others genotypes (around 2% less). Those results indicated also a strong variation for the same genotype from one year to another.

Table 1: **C18:3 values (in % of total fatty acids) for the most important varieties during three years.**

Varieties	2004	2005	2006
Aviso	10.3	10.1	8.6
Banjo	9.9	9.8	-
Campala	9.1	8.6	7.6
Grizzly	9.2	9.8	8.6
Pollen	6.8	7.2	-
Expert	8.3	8.5	-
Exocet	-	-	8.5
Quattro	-	-	7.1
Exagone	9	9.7	7.6

Location effect

In 2004, (Table2) the seeds collected in the East area get oil with a higher content in C18:3.

In 2005, the best composition was obtained with the seeds from the Centre of France and in 2006 it was with the seeds from Atlantic border.

Table 2: C18:3 average values (in % of total fatty acids) in 3 locations for the most important varieties during three years.

Location	2004	2005	2006
Atlantique border	7.4	9,1	8.6
East	9.5	8,9	7.7
Centre	8.9	9,6	7.8

Those results clearly indicated that the genotype effect, allied to the location were not the good criteria for explaining the fluctuation of the oil content in linolenic acid.

Effect of low temperatures

For each samples, we calculated the cumulated minimum temperatures during the sensitive period.

The figure 2 illustrated all the results obtained during 3 years for the 3 locations. In order to prevent from too strong genotype effect, we don't take into account the values for *Pollen*. There is a clear evidence of the relation between the low temperature during the sensitive period and the final content in linolenic acid of the rapeseed collected. If 9% value is expected in the oil for C18:3 content, this will be obtained through locations or years where the cumul of the low temperatures during 60 days after anthesis will be below 450°C. Over this threshold, the linoleic content will decrease, mainly by increasing the oleic and linoleic fraction, as indicated in figure1.

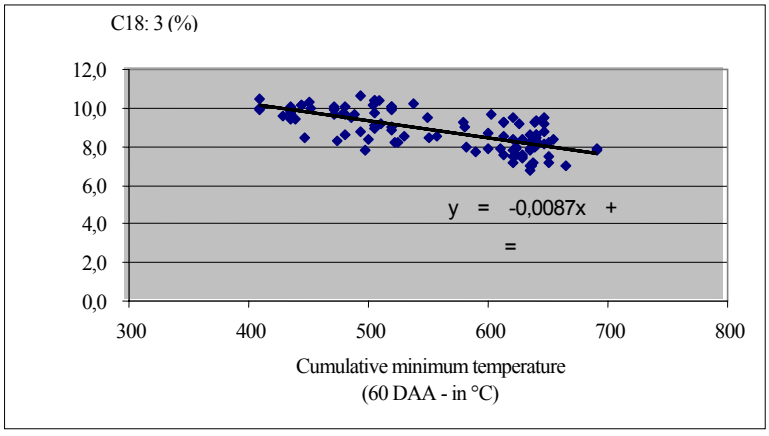

Figure 2: Relations between the cumulative minimum temperature 60 DAA (days after anthesis) and the C18:3 content in the rapeseed oil collected from different location over 3 years.

Discussion

Among the factors involved in fluctuation of fatty acid quality in oilseed rape the climatic conditions explain the difference between year and location. To secure a 9% threshold, crops needs to be grown in area with low temperature : no more than 450°c minimum temperature cumulated during 60 days after flowering.

The linear model fits quite well with the data. The fluctuation around the curve could came from climatic datas : variation could be obtained between the location of the climatic station and the field plots.

Nevertheless, with such a model, crushers could selected one month before harvest the best area where the threshold will be below 450°c. In this case, they could get a high probability to collected seeds with oil content in C18:3 over 9 %.

References

Champolivier, L., et Merrien, A., **1993** : Cinétique d'accumulation des teneurs en acides gras chez une variété de colza érucique. CR expérimental CETIOM-CBA ST Pathus, 08/1993, 9 pages.

Deng, X., Scarth, R., **1998** : Temperature effects on fatty acid composition development of low-linolenic oilseed rape (Brassica napus L.). *J.A.O.C.S.*, Vol. 75, n° 7, p. 759-766

Izquierdo, N. et al. **2002**: Night temperature affects fatty acid composition in sunflower oil depending on the hybrid and the phenological stage. *Field Crops research,* 4078, 1-12

Merrien, A., Pouzet, A., Krouti, M., Dechambre, J. et Garno, V. **2005** : Contribution à l'étude de l'effet des températures basses sur la composition en acide gras de l'huile des akènes de tournesol (oléique et classique). *OCL***,** vol.12, p.455-458.

Tremolières, H.A., Tremolières, A and Mazliac, P., **1978** : Effects of light and temperatures on the FA desaturation during the maturation of rapeseed. *Phytochemistry,* 17 : 685-687.

Biological activity of glucosinolate derived compounds isolated from seed meal of Brassica crops and evaluated as plant and food protection agents

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Abstract

Glucosinolates are amino acid derived allelochemicals characteristic of plants of the order Capparales. These compounds are present in seeds of agriculturally common Brassica crops in varying amounts and mixture profiles depending on the plant species. Glucosinolates are hydrolysed by endogenous enzymes (myrosinases; EC 3.2.1.147) and a variety of biologically active products are produced, with different structures depending on the parent glucosinolate and the reaction conditions. Isothiocyanates and their derivatives (e.g. oxazolidine-2-thiones), nitriles, epithionitriles, and the different products derived from indol-3-ylmethylglucosinolates have fungicidal, nematocidal and herbicidal effects and therefore their potential use as biodegradable natural products for crop protection has attracted much attention in the last years. In the present study, we have evaluated the effects of some glucosinolate transformation products on a number of plant pathogenic fungi. Different concentrations of glucosinolate hydrolysis products both individually and in combination were tested. All glucosinolate products were active against the different fungi and active concentrations ranged from 10 μM to over 10 mM. Glucosinolate products were shown to be specific depending on the fungi tested; therefore the targeted control of plant pathogens through the use of glucosinolates is possible. The use of glucosinolate concentrates or isolates could furthermore contribute to increase the value of the Brassica seed meal.

Introduction

Glucosinolates are a group of allelochemicals with well defined structures (Sørensen, 1990, Sørensen, 2001; Bellostas et al., 2007) characteristic of plants of the order Capparales. More than 140 structurally different glucosinolates are known (Bellostas et al., 2007) and about 10% are present in seeds of agriculturally common Brassica crops, although in varying quantities depending on the species (Bellostas et al., in press). The use of the remaining seed cake after oil extraction has traditionally been limited by the concentration of these compounds (Bellostas et al., this conference), however, the extraction of glucosinolates from seed meal is nowadays possible and it further contributes to an increased quality of the seed meal for feed (Bagger et al., this conference). Glucosinolates are hydrolysed by endogenous enzymes (myrosinases; EC 3.2.1.147) and a number of compounds are produced depending on the parent glucosinolate and the environmental conditions (Sørensen, 1990, Bellostas et al., 2007). Among these compounds, 5-vinyl-oxazolidine-2-thione is known for their antinutritional effects on monogastric animals, whereas isothiocyanates are fungicidal, nematocidal and herbicidal (Brown and Morra, 1997). Isothiocyanates are very reactive compounds that can easily react with nucleophiles present in the environment, such as free amino groups or thiol groups. Their reaction with free amino groups gives rise to thioureas, whereas reacting with thiol groups produces dithiocarbamates (Figure 1). At acidic pH and in the presence of redox co-factors such as Fe^{2+}, glucosinolate hydrolysis yields also nitriles, epithionitriles and thionamides (Bellostas et al., this conference), which are more hydrophilic and stable than isothiocyanates (Sørensen, 1990). The possibility for using glucosinolates as precursors for environmental friendly biocides therefore exists and it could contribute to increase the value of the Brassica seed meal.

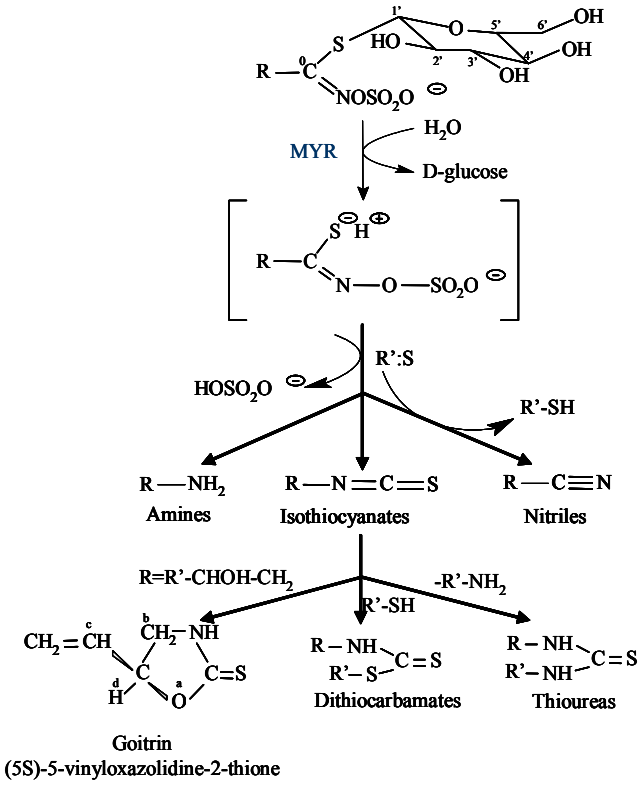


Figure 1. Hydrolysis of glucosinolates by myrosinase gives rise to a number of degradation products. Isothiocyanates are very reactive compounds and their reaction with nucleophiles gives rise to dithiocarbamates (reaction with thiol groups) and thioureas (reaction with free amino groups).

Materials and methods

Glucosinolates and myrosinases were isolated by laboratory standard procedures as well as in pilot plant scale (Bagger et al., this conference). Isothiocyanates and oxazolidine-2-thiones were prepared by hydrolysis of the corresponding parent glucosinolates in 100 mM phosphate buffer pH 6.5. Thioureas were produced by allowing the corresponding isothiocyanates to react with ammonia in an ethanol solution. Concentration and purity of these compounds was determined spectrophotometrically and by capillary electrophoresis. Nitriles were purchased from Sigma-Aldrich (St. Louis, MO). The compounds were diluted in an emulsion of hydrolysed rapeseed oil (Sørensen, 2001) and afterwards added to cooled (50-60ºC) PDA medium (0.5 % v:v). Different formulas were developed, including the compounds individually and in combination. *Pseudocercosporella herpotricoides*, *Botrytis cinerea*, *Rhizoctonia solani, Fusarium oxysporum Aphanomyces euteiches* var. *pisi*, *Gaeumannomyces graminis* var. *tritici* and *Verticillium dahliae* were the fungi chosen due to their economic importance. Control Petri dishes consisted of the PDA medium with emulsion (0.5% v:v). The tests were performed at the Danish Institute of Agricultural Sciences at Flakkebjerg, at Grønt Center in Holeby and at Inabonos, Spain following their standard methods.

Results and discussion

The glucosinolate hydrolysis products tested showed very different inhibition potential depending on the fungi and the structure of the degradation compounds, both regarding their side chain but also in relation to the type of degradation product (isothiocyanate, nitrile or thiourea). Figure 2 shows an example of how a single fungus (*Pseudocercosporella herpotricoides*) was differently inhibited by six types of degradation products, three nitriles (left) and three isothiocyanates (right). The side chains of these compounds correspond to three different parent glucosinolates: sinigrin (prop-2-enylglucosinolate), glucotropeolin (benzylglucosinolate) and gluconasturtiin (phenethylglucosinolate). Sinigrin is the dominant glucosinolate in Brassica mustards (*B. nigra, B. carinata* and *B. juncea*), in which it can be present in concentrations of up to 200 umol g dm^{-1} (Bellostas et al., in press).

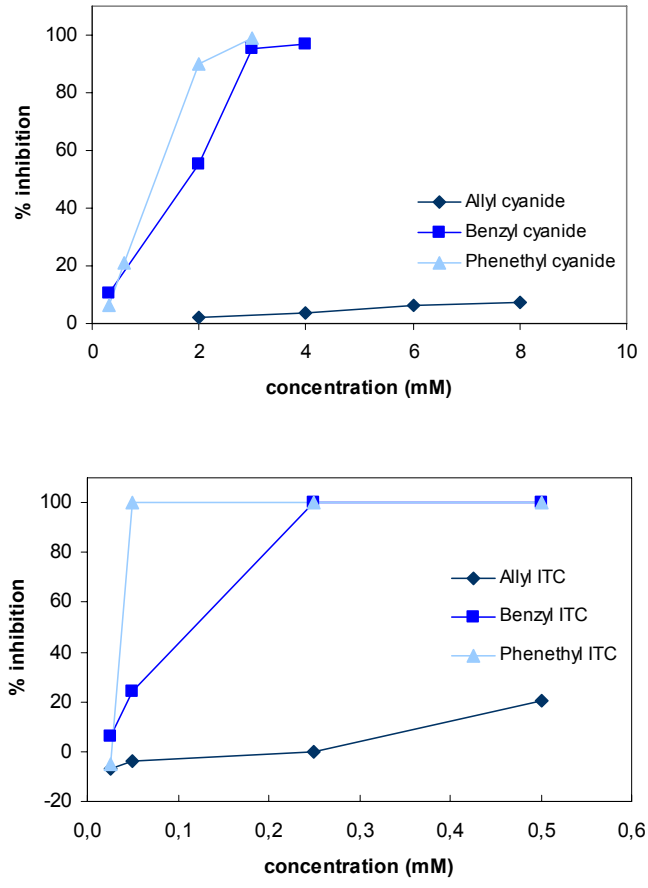
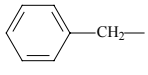
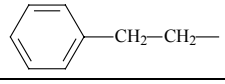


Figure 2. Effect of allyl, benzyl and phenethyl nitriles (left) and isothiocyanates (right) on *Pseudocercosporella herpotricoides*, a wheat soil-borne pathogen.

The three nitriles tested showed much lower activity than the isothiocyanates, as the concentration needed to inhibit the growth of the fungus was up to 10 times higher. Both the nitrile and the isothiocyanate derived from the allylglucosinolate showed the lowest inhibition potential of the fungus, whereas the nitrile and isothiocyanate from phenethylglucosinolate showed the highest inhibition potential. Benzyl nitrile and –isothiocyanate showed an intermediate activity, closer to that of phenethylglucosinolate derived products, as the structure of its side chain is very similar to this glucosinolate (Table 1).

Table 1. Semisystematic name and structure of side chain of the three glucosinolates tested against *Pseudocercosporella herpotricoides* (Figure 2).

Glucosinolate name	Structure of side chain
Prop-2-enylglucosinolate	$CH_2=CH—CH_2—$
Benzylglucosinolate	
Phenethylglucosinolate	

Conclusions

The glucosinolate hydrolysis products tested showed a high degree of specificity, being very toxic against some targets, while innocuous towards other organisms. This can be explained by the fact that high hydrophobicity of the side chain of a compound can confer it the ability to penetrate membranes. The possibility therefore exists for a selective control of plant pathogens while respecting the non-target organisms. Despite their lower toxicity compared to isothiocyanates, nitriles are generally more soluble in water, which may allow them to persist longer in soils. Nitriles are produced at low pH and in the presence of thiol groups and ferrous ions, which can be quite common in soils, therefore, the role of nitriles in biofumigation should also be considered.

Acknowledgement

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References

Bellostas, N., Sorensen, J.C., Sorensen, H. Profiling Glucosinolates in Vegetative and Reproductive Tissues of Four *Brassica* Species of the U-Triangle for Their Biofumigation Potential. Journal of the Science of Food and Agriculture. In press.

Bagger, C., Bellostas, N., Jensen, S. K., Sørensen, H., Sørensen, J. C., Sørensen, S. Processing-bioprocessing of oilseed rape in bioenergy production and value added utilization of remaining seed components. This conference.

Bellostas, N., Bjergegaard, C., Jensen, S. K., Sørensen, H., Sørensen, J. C., Sørensen, S. Nutritional value of cruciferous oilseed crops in relation to profile of accumulated biomolecules with especial regard to glucosinolate transformation products. This conference.

Bellostas, N., Sørensen, A.D., Sørensen, J.C. and Sørensen, H.: Genetic variation and metabolism of glucosinolates in cruciferous oilseed crops. In: Rapeseed Breeding: Advances in Botanical Research (Ed. Dr. Surinder Gupta) Academic Press/ Elsevier, Dan Diego, Vol. 54; 2007.

Brown PD, Morra MJ, Control of soil-borne plant pests using glucosinolate containing plants, *Advan. Agron.* 61, 167-231 (1997).

Sorensen, J. C., Ph.D. Thesis, Kongelige Veterinær-og Landbohøjskole, Copenhagen, 2001.

Sørensen H, Glucosinolates: Structure – Properties – Function, in *Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology*, ed. by Shahidi. F. Van Nostrand Reinhold Publishers, New York, pp. 149-172 (1990).

Analysis of the lignin contents and related enzymes activities in seed coat between black-seeded and yellow-seeded rapes (*Brassica napus*)

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Abstract

A pair of near isogenic yellow/black seeded rape (*Brassica napus*) were used as experimental materials to study the changes of lignin contents and enzymes activities of 4-coumarate: CoA ligase (4CL), Cinnamyl alcohol dehydrogenase (CAD) and ferulate 5-hydroxylase (F5H) in seed coat during seed development. The results showed that the changes of lignin contents and enzyme activities of 4CL, CAD and F5H in seed coat had significant differences between black- and yellow-seeded rapes, and also between different development stages. Correlation analysis demonstrated that the lignin contents were positively related to the activities of all three enzymes investigated in the study, and the interactions between them in the seed coat of the two lines. For yellow-seeded rape, except the correlation coefficient of lignin content and 4CL was not significant, the other correlation coefficients were significant or even highly positively significant. For the black-seeded rape, only the lignin content was highly positively related to the activity of F5H, the other correlation coefficients were not significant. It is suggested that 4CL, CAD and F5H regulate the biosynthesis of lignin in the seed coat of the rapes, leading to the lignin contents in the seed coat of the yellow-seeded rape much lower than that of the black-seeded line, and affecting the thickness of seed coat in rapes.

Key words: *Brassica napus*, Lignin contents, 4CL, CAD, F5H

Introduction

The yellow-seeded rape has been one of the major foci in breeding research. Because yellow-seeded rape has lower seed coat ratio, higher oil content and more transparent oil than their black-seeded counterpart. However, it is hard to obtain genetically stable and true breeding lines of yellow-seeded types in *B. napus*. In recent years, great attentions have been paid to the mechanisms of its seed coat formation. Previous studies have shed light on the understanding of the biochemical mechanism of seed coat formation in yellow-seeded rapes. It has been found that polyphenols, anthocyanin, flavonoid and melanin contribute to the color and lustre in seed coat of black- and yellow-seeded rapes (Ye et al., 2002), and it is implied that anthocyanin and flavonoid were break down or transformed into the precursors of melanin biosynthesis during the late stage of the seed development (Ye et al., 2002).

It is well known that related substrates and enzymes are required for the pigments biosynthesis, and the enzymes of polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) in seed coat were found to be very important effect on the forming of the seed color of rapes (Wang et al., 1991; Ye et al., 2001, 2001); and it was implied that PAL, PPO and peroxidase (POD) affected the thickness of the developing seedcoats in rape by regulating the biosynthesis of lignin (Liang, et al., 2004).

Lignin is a complex polymer formed by the oxidative polymerization of hydroxycinnamyl alcohol derivatives termed monolignols, and 4CL, CAD and F5H were believed to be key enzymes involved in lignin biosynthetic (Boudet et al., 1995; Baucher et al., 1998). It was reported that lignin was one of the key factors that lead to the lower coat ratio in the seed coats of the yellow-seeded rape than that of the black-seeded rape (Liang et al., 2002). In order to explain the different roles of enzymes played in the leading of lower lignin contents in the seed coats of the yellow-seeded rape, and to better understand the mechanism of seed coat character's formation of yellow-seeded and black-seeded rapes with attempt to provide the theoretical assistance for the yellow-seeded rape breeding, the lignin contents and the three key enzymes activities were investigated, and the changes of lignin contents, related enzymes activities of 4CL, CAD and F5H were analyzed in seed-coats during seed development between black- and yellow-seeded rapes by near iso-genic lines.

Materials and Methods

A pair of near iso-genic line of rapes (*Brassica napus*), L1 (black-seed) and L2 (yellow-seed), were the descendants derived from the cross combination of *Brassica napus* and *Brassica juncea*. In 1995, an individual plant of black-seeded rapeseed was identified in the tenth progeny of the yellow inbred lines, which was identical to the yellow seeded one in both growth and appearance. The desirable plants were obtained in the third generation of the black inbred line by inbreeding the black-seeded individual plant, which was identical to the yellow seeded one except that the seed color was black. The stable inbred lines of black and yellow seeded rape seeds formed the near-isonegic pairs. In order to maintain genetic consistency of the near iso-genic lines, the offsprings were preserved by using the black-seeded individual plant as recurrent parent and adopting the BC_1 and F_1. The seed coats were sampled at 20, 25, 30, 35, 40, 45d till to the full maturation of seed at 50d after

flowering. All the analyses were performed in triplicate. The triplicate of L1 was marked L1-1, L1-2 and L1-3, and that of L2 was marked L2-1, L2-2 and L2-3.

Lignin contents were analyzed according to previously described protocols (Xing, 1981). Dried seed coats (0.5 g) were used for the analysis. After removing the concomitants, the lignin was oxidized by the $K_2Cr_2O_7$ with the H_2SO_4.

Enzyme extraction and the enzymes activities assays: the enzyme extraction was using 0.1 mol L-1 Tris-HCl buffer (pH 7.5; 20 mmol L^{-1} mercaptoethanol; and 5% [w/v] polyvinylpolypyrrolidone), protein concentrations were determined using the Bio-Rad reagent (Bradford, 1976). 4CL activity was measured at room temperature by spectrophotometric assay to detect the formation of CoA esters of *p*-coumaric acid (Knobloch et al., 1977). CAD activity assays were carried out following Abbott's protocol (Abbott et al., 2002) with some modification. For F5H activity was determined by a NADPH regenerating system (Humphreys et al., 1999).

The data was analyzed with the DPS statistical software.

Results

As showed in Fig.1, the lignin contents reached to its maximum level when the seeds had grown for 40 days in the 2 lines of rapes. The maximal lignin content of the black-seeded rape was 13.56% while that of the yellow-seeded rape was only 4.92%. When the seeds nearly got ripen at 50 days, the lignin content of the yellow-seeded rape (3.15%) was a little higher than that (2.41%) in the early seed development stage, but that of the black-seeded one (13.32%) was much higher than that (5.94%) in the early stage.

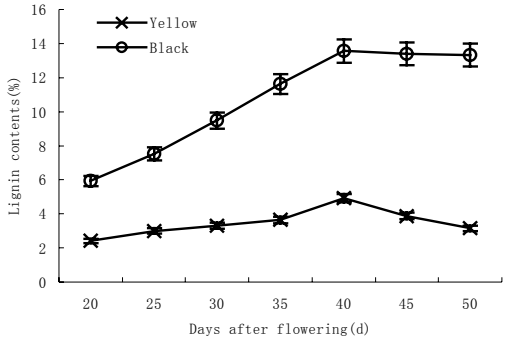

Fig.1 Changes of lignin content in seed coat

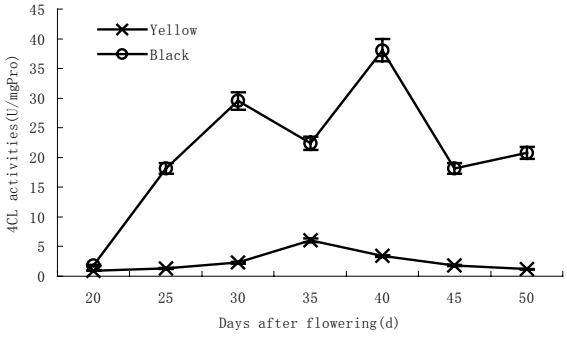

Fig.2 Changes of 4CL activities in seed coat

The changes of 4CL activities in seedcoats of the yellow-and black-seeded rapes were shown in Fig.2. In general, the 4CL activities of the two species increased at first, then decreased and increase again. The change of black-seeded rape is sharper than that of the yellow-seeded one. Enzyme activity of black-seeded reached its maximum level (38.09 U mg^{-1}Pro) at 40 days, and that of yellow-seeded one reached the maximum (6.10 U mg^{-1}Pro) at 35 day. The black one reached the second peak (29.55 Umg^{-1}Pro) at 30 days.

As showed in Fig.3, CAD activities of the two species firstly rose, and reached maximum at 40 days (Yellow-seeded rape: 0.2290 U mg-1Pro, Black-seeded rape: 1.4083 U mg-1Pro), then fell. But the enzyme activities of the black-seeded one increase significantly at 30 days and then dropped suddenly after reached its maximum; however the enzyme activity of the yellow-seeded rapes had two peaks including the maximum peak value, and begun to increase 5 days latter than the black one (Fig.3).

Fig. 4 demonstrated the changes of F5H activities in seedcoats of yellow-seeded and black-seeded rapes. The patterns of the F5H activity of the two NILs were different. The activity of F5H in the black-seeded rape reached its peak (1.8119 U $^{-1}$mg Pro) at 45 d while it reaches its peak at 40 days in the yellow one (0.2285 U mg-1 Pro).

As showed in table 1, in the seedcoats of the yellow-seeded rape, besides the correlation coefficient (0.4964) of lignin content and 4CL activity was not noticeable, the correlation coefficient (0.7262) of lignin content and the interaction between 4CL and F5H was noticeable, and the lignin contents were marked positive correlative to the activities of CAD and F5H, the interaction between 4CL and CAD, and the interaction between CAD and F5H in seedcoats of the yellow-seeded rape, the correlation coefficients of them were 0.9213, 0.9150, 0.8907, and 0.9214 respectively.

As showed above in table 2, in the seedcoats of the black seeded rape, only the lignin content was noticeable correlative to the activity of F5H, and the correlation coefficient was 0.772949, the other correlation coefficients of lignin contents to 4CL, CAD activities, the interactions between the three enzymes were 0.672363, 0. 541259, 0.503105, 0.703452, and 0.531204 respectively, they were not noticeable, but they all were above 0.5000.

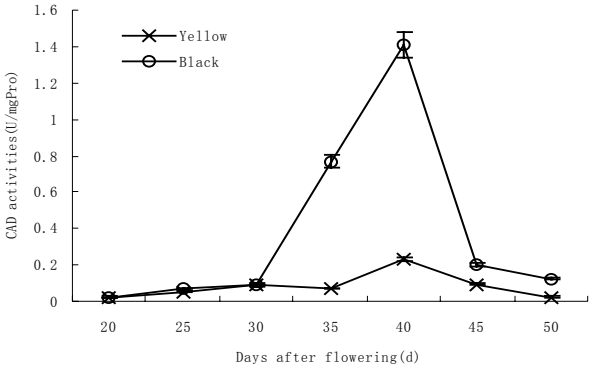

Fig.3 Changes of CAD activities in seed coat

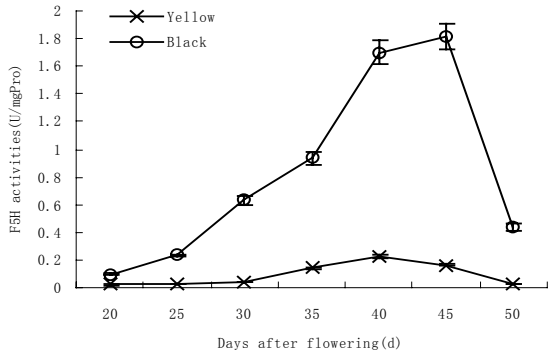

Fig.4 Changes of F5H activities in seed coat

Table.1 The correlation coefficients of lignin contents, 4CL, CAD and F5H activities in seed coat of yellow- seeded rape

Correlation coefficients	4CL	CAD	F5H	4CL × CAD	4CL × F5H	CAD × F5H	Lignin contents	Significant levels
4CL	1.0000	0.3725	0.5808	0.6869	0.8997	0.3648	0.4964	0.2571
CAD	0.3725	1.0000	0.8327	0.9124	0.6565	0.9601	0.9213	0.0032
F5H	0.5808	0.8327	1.0000	0.8576	0.8522	0.8834	0.9250	0.0028
4CL × CAD	0.6869	0.9124	0.8576	1.0000	0.8833	0.9106	0.8907	0.0071
4CL × F5H	0.8997	0.6565	0.8522	0.8833	1.0000	0.7069	0.7620	0.0465
CAD × F5H	0.3648	0.9601	0.8834	0.9106	0.7069	1.0000	0.9214	0.0032
Lignin contents	0.4964	0.9213	0.9250	0.8907	0.7620	0.9214	1.0000	1E-08

Table.2 The correlation coefficients of lignin contents, 4CL, CAD and F5H activities in seed coat of black- seeded rape

Correlation coefficients	4CL	CAD	F5H	4CL × CAD	4CL × F5H	CAD × F5H	Lignin contents	Significant levels
4CL	1.0000	0.6782	0.6278	0.6998	0.7770	0.6880	0.6724	0.0980
CAD	0.6782	1.0000	0.6424	0.9774	0.8359	0.9719	0.5413	0.2096
F5H	0.6278	0.6424	1.0000	0.6259	0.9133	0.6889	0.7729	0.0416
4CL × CAD	0.6998	0.9774	0.6259	1.0000	0.8645	0.9951	0.5031	0.2498
4CL × F5H	0.7770	0.8359	0.9133	0.8645	1.0000	0.8999	0.7035	0.0778
CAD × F5H	0.6880	0.9719	0.6889	0.9951	0.8999	1.0000	0.5312	0.2199
Lignin contents	0.6724	0.5413	0.7729	0.5031	0.7035	0.5312	1.0000	1E-08

Discussion

The seed coat color development are well documented (Ye et al., 2002, 2002), some enzymes involved in pigment biosynthesis have also been reported (Wang et al., 1991; Ye et al., 2001, 2001); it is reported that the lignin was one of the key factors that leaded to the lower coat ratio in seed coats of the yellow-seeded rape than that of the black seeded one (Liang et al., 2002), and it was implied that PAL, PPO and POD affected the thickness of the developing seedcoats in rape by regulating the biosynthesis of lignin (Liang, et al., 2004). 4CL, CAD and F5H are considered as key enzymes participating in lignin biosynthesis (Liang, et al., 2004). Therefor, it was anticipated that the lignin components might be different between yellow- and black-seeded rapes, and the S/G of the yellow-seeded rape were lower that of the black one, the activity of 4CL caused great difference in lignin contents, it was CAD and F5H leaded the quantitative difference of G- and S- monolignol between yellow- and black-seeded rapes. So, it was feasible to change the seedcoat ratio by over expressing or suppressing the activities of theses three enzymes or one of them. Whether the difference of the enzymes in the two species was due to DNA or RNA or even protein changes is still unknown, it needs to be further investigated by utilizing molecular biology or biotechnology techniques.

Hence, it is necessary to investigate the lignin biosynthesis, this is useful not only for elucidating the characters of the seed coat of rapes, but also the lignin biosynthetic mechanism in various cell types, tissues and organs of different genetic rapes. It is feasible to cultivate new kinds of rapeseed, which can not only provide more qualified oil but also become more adaptive to kinds of stresses by bioengineering methods in combination with conventional breeding techniques.

References

Abbott J C, Barakate A, Gaelle Pinc¸on, Legrand M, Lapierre C, Mila I, Schuch W, Halpin C. Simultaneous suppression of multiple genes by singletransgenes.

Down-regulation of three unrelated ligninbiosynthetic genes in tobacco. Plant Physiology, 2002, 128: 844-853.
Baucher M, Monties B, Van Montagu M, Boerjan W. Biosynthesis and genetic engineering of lignin. Crit Rev Plant Sci, 1998, 17: 125-197.
Bradford M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 1976, 72: 248-254.
Boudet A M, Lapierre C, Grima-Pettenati J. Biochemistry and molecular biology of lignification. New Phytol 1995, 129: 203-236.
Humphreys J M, Hemm M R, Chapple C. New routes for lignin biosynthesis defined by biochemical characterization of recombinant ferulate 5-hydroxylase, a multifunctional cytochrome P450-dependent monooxygenase. Proc. Natl. Acad. Sci. USA, 1999, 96: 10 045-10 050.
Knobloch K H, Hahlbrock K. 4-coumarate: CoA ligase from cell suspension cultures of Petroselinum hortense Hoffm. Archives of Biochemistry and Biophysics, 1977, 184: 237-348.
Liang Y, Li J N. The relationship of color formation with related enzymes and protein contents in the seedcoat of oilseed rape (Brassica napus). Agricultural Science in China, 2004, 5: 384-391.
Liang Y L, Liang Y, Li J N, Chen L. Difference between yellow and black seed coat in B. napus L..Chinese Journal of Oil Crop Sciences, 2002, 24: 14-18.
Wang H Z, Liu H L. Histochemical research of polyphenol oxidase (PPO) in seed coat and the relationship between ppo and seed color in rapeseed. Chinese Journal of Oil Crop Sciences, 1991, 13: 30-32.
Xing J H. Plant Biochemical Analysis. Science Publishing Company, 1981: 158-181.
Ye X L, LI J N, Tang Z L, Liang Y, Chen L. Study on seed coat colour and related characters of Brassica napus L.. Acta Agronomica Science, 2002, 27: 550-556.
Ye X L, Li X G, Li J N. Mechanism of melanin synthesis in seed coat of Brassica napus L.. Acta Agronomica Science, 2002, 28: 638-643.
Ye X L, LI J N, Tang Z L,Chen L. Difference of seed coat color between black and yellow -seeded in B.napus with seed development: Changes of anthocyanin, phenylalanine and phenylalanine ammonia-lyase and their correlation analyses. Chinese Journal of Oil Crop Sciences, 200l, 23: 38-45.
Ye X L, LI J N, Tang Z L, Chen L. Difference of seed coat color between black and yellow -seeded in B.napus with seed development ¢ò Changes of melanin and tyrosine and tyrosinase and their correlation analyses. Chinese Journal of Oil Crop Sciences, 200l, 23: 38-45.

The dynamics of the bioactive compounds in oilseeds

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Abstract

Plant sterols have gained lot of interest in recent years. It is mainly because of their cholesterol-lowering effect in human blood serum, therefore they are used as active compounds in medicaments for cardiovascular diseases. Various other important applications, for example cure for some forms of cancer, are also known. Sterols are not only manufactured as medicaments but also as active ingredients in various functional foods. There's an ongoing search for rich sources of natural sterols. Sterols from wood industry's tall oil and soya oil deodorisation destillate have mostly been used so far. The obtained data could be used to acknowledge rapeseed not only as a good source of oil and animal feed but also for bioactive compounds.

The goal of our research was to determine the phytosterol and tocopherol level in locally grown rapeseed oil to evaluate its potential as a valuable source for bioactive compounds besides healthy food component. Moreover, the object of this study is to investigate the effect of various factors on the content and composition of said compounds.

Key words: rapeseed oil, fertilizers, sterol, tocopherol.

Introduction

Rape (*Brassica napus* L.) is now the third most important source of vegetable oil in the world. While the growing area of rapeseed in Estonia has increased tremendously in last two decades, yields have remained relatively low. Low yields are mainly due to misjudgements in agrotechnical principles (Kaarli, 2004).

Rapeseed is getting more popular among Estonian farmers besides linseed, hempseed and golden flax. Its agronomic and economic value is widely known. Besides two main products, oil and meal, rapeseed is also good source of bioactive compounds – phytosterols and tocopherols, containing about 0,5-1,2% of them in oil. It is well-known fact that the main sterol, sitosterol has cholesterol-lowering effect in blood serum and is therefore used as active compound in treatment of cardiovascular diseases. Numerous other medical and non-medical properties are also known (Moreau *et al.,* 2002; Quilez, 2003). Tocopherols are widely recognized as naturally occurring antioxidants. There's a everlasting search for rich sources of natural bioactive chemicals. Sterols from wood industry's tall oil and soya oil deodorisation destillate and tocopherols from various oilseeds have mostly been used so far.

Various scientists have analyzed the sterol composition of rapeseed oil but only few articles have been published on different factors affecting the sterol composition in plants. The obtained data could be used to acknowledge rapeseed not only as a good source of oil and animal feed but also various bioactive compounds. Nevertheless, lot of authors have mentioned that genetic backround, fertilization, weather parameters, growing medium as well as some oil refining steps could have possible effect on sterol content and composition. Therefore, information about the phytosterol and tocopherol content in local rapeseed oil is needed and due to emergence of many small-scale cold-pressing oil enterprises where the origin of the seeds could be detected, this information becomes more important.

The goal of our research was to determine the phytosterol and tocopherol level in locally grown rapeseed oil samples to evaluate its potential as a valuable source for bioactive compounds besides healthy food component. Moreover, the object of this study is to investigate the effect of various factors on the content and composition of said compounds.

Materials and Methods

In order to investigate the phytosterol and tocopherol content in oil of samples, cultivars were used from field trials at the Department of the Field Crop Husbandry at EMU and also National Testing Centers (3). Samples of other oilseeds were from trial fields of Jõgeva Plant Breeding Institute. For the the analysis of different fertilizers, the trial was carried out in 4 replications and the size of plots was 10 x 1 m. The soil type was pallescent soil LP (Kõlli & Lemetti, 1994), a glossisol by FAO classification and a Stagnic Luvisol by WRB classification (Deckers *et al.,* 1998). The trial soil was neutral – pH_{KCl} 6.2; humus 2.4%, available phosphorus 77.7 mg kg^{-1} (AL); mobile potassium 169.8 mg kg^{-1} (AL); calcium 5,648.0 mg kg^{-1}, sulphur 13.5 mg kg^{-1} of the soil. The field was fertilized with mineral complex granular combined fertiliser OptiCropNPK 21-08-12-S-Mg-B-Ca, calculating 120 kg of the active substance agent of nitrogen per hectare. In variants different microfertilizers were used.

Sterol analysis. Method for phytosterol analysis was worked out by using and optimising analytical steps known from literature. Total sterols and sterols in free form were determined. For quantification of sterols, internal standard method was used. Sterol content and composition was detected over a 3-year period (2001-2003) and 6 different microfertilizers were used as variants. Three major sterols (β-sitosterol, campesterol, brassicasterol) which take up about 90% of all the plant sterols in

rapeseed oil, were determined. As sterols occur in vegetable oils in free form or bound with fatty acids, the amount of both forms was calculated.

Sample treatment. For total sterol analysis, the dried seeds were cold-pressed and oil samples (each 500 mg) were taken in 3 replications. 200 µl of internal standard solution (500 mg of cholesterol in 100 ml ethanol) was added. After addition of 0.5 ml KOH solution (60% in water) and 4.5 ml ethanol, the oil samples were hydrolyzed for 45 minutes at 70 °C. Then 3 g of silica was added and the reaction mixture was evaporated to dryness. 1 g of Na_2SO_4, 1 g of silica and 3 g of silica with the sample were loaded onto glassfilter and eluated with 20 ml of ethyl acetate/diethyl ether mixture (1:1). The solvents were evaporated, 1 ml of dichloromethane was added. 1 µl of sample mixture was injected into gas chromatograph.

For analysis of tocopherols and sterols present in free form, oil samples of 250 mg were taken. 200 µl of cholesterol in ethanol and 3 ml of dichloromethane were added. 10 ml solid-phase extraction (SPE) cartridge, filled with 30 µm silica, was conditioned with 10 ml hexane/ethyl acetate mixture (80:1) and 3 ml of sample was loaded. Then, the cartridge was eluted with 20 ml hexane/ethyl acetate mixture (80:1), 30 ml (20:1) and finally the sterol fraction was collected with 20 ml hexane/ethyl acetate mixture (3:1). The solvents were evaporated, 1 ml of dichloromethane was added. 1 µl of sample mixture was injected into gas chromatograph.

Gas chromatography. GC analysis was performed on HP5890 instrument, using 25 m x 0.25 mm, i.d. 0.22 µm, BP-5 fused silica capillary column. Nitrogen was used as carrier gas. The temperature was 300 °C, total time 20 min. Injector 300 °C, flame-ionization detector 340 °C.

Results and discussion

Various rapeseed varieties from national testing centers and also from the trial fields of Estonian University of Life Sciences were used. Method containing oil sample clean-up and subsequent quantification by gas chromatography was worked out. Also the precision of sterol analysis was evaluated.

The limit of detection (LOD, S/N ratio > 3) and limit of quantification (S/N ratio > 10) were determined on the basis of sitosterol. LOD of the GC signal of sitosterol was 5 µg/ml and LOQ was fixed at 62 µg/ml. The average sitosterol content that was determined over a 4-month period twice a week, was 396.9 mg/100 g oil (relative standard deviation 1.09%), average campesterol content was 315 mg/100 g oil and 81.1 mg for brassicasterol, relative standard deviations were 1.15% and 1.17%, respectively.

Different seed samples were further characterized by their oil phytosterol content. In present research the effect of weather and fertilizers on plant sterol content and composition was evaluated. The total sterol content of oil samples varied between 5220 and 6550 mg kg^{-1}, the main sterol being β-sitosterol followed by campesterol and brassicasterol.

The amount of sterols present is decided by the intensity of their biosynthesis and their role in the plant cell. Free sterols are present in cell membrane bilayer where they regulate the fluidity of the membrane and therefore also the transport of different compounds through the cell. Steryl esters are probably the deponated forms of sterols inside the cell. It is also known that plants use phytosterols for adaptation to different temperatures (Piironen *et al.,* 2000).

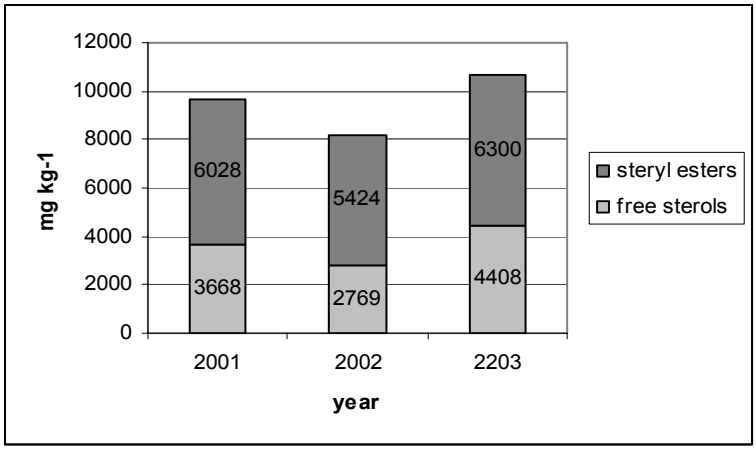

Fig. 1. The sterol content (as free sterols and steryl esters) of the oil from spring rapeseed variety's 'Mascot' in 2001-2003, mg kg^{-1}.

It was found that weather during the growing cycle of rapeseed had a clear impact on sterols. Summer in 2002 was extremely dry and hot, totally opposite to the vegetation period in 2003, while summer in 2001 had about average weather conditions. It was found that the total sterol content varied between 5220-5770 mg kg^{-1} in 2002 and 5910-6550 mg kg^{-1} in 2003 (Figure 1). It is rather difficult to assess the influence of different weather factors to the sterol content. It is known that the sterol levels could change drastically due to long drought period in some plants but linear interpolations could not be made for less extreme weather conditions.

There are couple of articles about the effect of temperature during growing period of soya on the total sterol content. Scientists have found a positive correlation of sterol and higher temperature values (Vlahakis *et al.,* 2000). Present research indicates rather the opposite. While in 2003 where average temperatures were lower than in two previous years, the total sterol content in oil samples was higher. This may be due to effect of other factors including precipitation, soil parameters,

fertilization, etc.

The ratio of free sterols to steryl esters is usually 1/3 to 2/3 according to Lampi *et al* (2004). The amount of different forms of sterols could change due to plants need to adapt to various conditions. Knowing the ratio is mainly necessary for manufacturing functional food products. Results obtained in present research fall into the category described in literature.

There are two important issues from the fertilization point of view. Does the fertilization which ensures good development of the rapeseed plant and high oil yield with good quality parameters also provide bigger amount of plant sterols? Does the fertilization with certain element(s) influence the sterol content and composition in plants? The effect of different microfertilizers on sterol levels was investigated during 3-year period. The dynamics of the main sterol (β-sitosterol) is given below (Table 1). Noticeable differences were observed but no certain influence could not be detected over research period. Correlations between oil yield and total sterol content was also not observed.

Table 1. Content of total β-sitosterol in different oil samples in 2001-2003, mg kg^{-1}.

Variant	β-sitosterol, mg kg^{-1}			Average
	2001	2002	2003	
0 (control)	-	2540	2970	2760
OptiCrop+Copper	2820	2630	2670	2700
OptiCrop+Boron	2690	2500	2760	2650
OptiCrop+Manganese	2690	2370	2890	2650
OptiCrop+Sulphur	-	2440	2890	2670
OptiCrop+Micro Rape	2430	2660	2730	2610
Average	2660	2520	2820	2670

No influence of various micronutrients on different forms of sterols and overall distribution of individual sterols was found.

Tocopherol content has been observed in 10 spring rapeseed cutivars so far. It has been found that only α- and γ-tocopherol have been found in rapeseed oil. Other isomers (β- and δ-tocopherol) were not found in determinable amounts. α- and γ-tocopherol contents varied, respectively 21-27 mg and 46-52 mg/100 g oil in samples of 2004.

It must be said that some of the findings are preliminary in nature and need to studied more deeply to be stated with more certainty. Nevertheless it was found that the weather of the trial years was the most influencing factor concerning the total amount of sterols. Also that spring and winter rapeseed varieties differ mostly in proportions of steryl esters and free sterols. Plant physiologs have suggested that plants tend to use its steryl esters as a defense mechanism against cold temperatures (Ferrari, 1997). Accordingly it was found that the oil of winter rapeseed varieties had significantly higher proportion of steryl esters. In general, the amount of different forms of sterols could change due to plants need to adapt to various conditions. Knowing the ratio of different forms of sterol conjugates is mainly necessary for manufacturing functional food products. Results obtained in present research for spring rapeseed variants fall into the category described in literature that ratio of free sterols to steryl esters is usually 1/3 to 2/3.

Influence of factors affecting sterol and tocopherol content and their content in other oilseeds in Estonia will be discussed in more detail.

Conclusions

There are several factors affecting the amount, proportions etc of said biochemical compounds that are of interest in light of making functional foods, biomedicines. Rapeseed is by far the best source of phytochemicals for industrial extraction in Estonia. It was found that mostly weather during growing period influenced the total content of sterols in rapeseed oil samples. The sterol content of oils from rapeseeds investigated where various fertilizers were used showed variation but the effect of different fertilizers on sterols could not been interpreted clearly. Various other preliminary findings were observed that need more studies.

References

Deckers, J. A. *et al* (Eds.) (1998) World Reference Base for Soil Resources. Introduction. ACCO Leuven, 165. p.

Ferrari, R. A. *et al* (1997) Alteration of Sterols and Steryl Esters in Vegetable Oils during Industrial Refining. Journal of Agricultural and Food Chemistry, 45, pp. 4753-4757.

Kaarli, K. (2004) Õlikultuuride kasvataja käsiraamat. Saku, OÜ Greif, 131 p. (in Estonian)

Kõlli, R. and Lemetti, I. (1994) Eesti muldade lühiiseloomustus I. - Normaalsed mineraalmullad, EPMÜ, Tartu: Trükokoda Tartumaa, 122 p. (in Estonian).

Lampi, A-M. *et al* (2004) Analysis of Phytosterols in Foods. In: Phytosterols as Functional Food Components and Nutraceuticals (Editor P. C. Dutta). New York, Marcel Dekker, Inc., pp. 33-70.

Moreau, R. A. *et al* (2002) Phytosterols, phytostanols and their conjugates in foods: strucutral diversity, quantitative analysis and health-promoting uses. Progress in Lipid Research, 41, pp. 457-500.

Piironen, V. *et al* (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. Journal of Science of Food and Agriculture, 80, pp. 939-966.

Quilez, J. *et al* (2003) Potential uses and benefits of phytosterols in diet: present situation and future directions. Clinical Nutrition, 22 (4), pp. 343-351.

Vlahakis, C. and Hazebroek, J. (2000) Phytosterol Accumulation in Canola, Sunflower and Soybean Oils: Effects of Genetics, Planting Location and Temperature. Journal of the American Oil Chemists' Society, 77 (1), pp. 49-53.

Studies on volatile compounds of different varieties in *Brassica napus* L.

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Abstract

To compare volatile components of different varieties, volatile compounds were extracted by water distillation approach from entire plants 25 days before maturation in *Brassica napus* L and assayed by capillary gas Chromatography-mass spectrometry (GC-MS). A total of 107 different components were identified. Volatile profile of four varieties Tapidor, Ningyou No.7, NJ5412 and Heza No.7 included 57,51,45 and 43 compounds, respectively. 15 compounds were common among the 4 varieties, including high-content phytol and oleic acid, also including β-damascenone,β-ionone and myristic acid, etc. Volatile profiles were quantitatively and qualitatively different among the varieties. In Tapidor, there were 2 kinds of isothiocynate with relative content 0.36%, but there were 7 kinds of isothiocynate with relative content 2.43% in Ningyou No.7. As to linoleic acid content, it was 18.55% in Ningyou No.7, but less than 2% in the others. As an exception, NJ5412 did not contain insect attractant cis-3-hexenol. Totally, different varieties had different volatile profiles that probably are basis of genotype selection.

Key words: *Brassica napus L*; volatile compounds; GC-MS

Introduction

The volatile compounds play crucial roles in whole growing period of plants. Plants release small amounts of diverse volatile blends including terpenoids, benzenoids, and fatty-acid derivatives, etc, accounting for about 1% of the plant secondary metabolites [1], but that little materials released function in many important aspects such as pollinator attraction, defense and communication [2]. Interestingly, by releasing volatiles, signaling plant can reduce number of herbivores more than 90% [3], and can also warn neighboring plants about the pathogen attack [4]. Recently, great progress has been made in biochemical and molecular characterization on mechanism of plant volatile formation and release. Research achievements imply that controlling release of special volatile compounds benefits well variety development through bioengineering or conventional approach.

In limited reports on *Brassica* volatiles, glucosinolate–myrosinase system (GLS-MYR), which produces special chemical mixtures in defense systems, plays very important roles in antagonizing biotic and abiotic stresses [5]. The main aim of this paper is to provide a preliminary screening of volatiles from different rapeseed varieties for revealing variety differences.

Materials and methods

Plant materials

Four varieties (line) of rapeseed, Tapidor, Heza No.7, Ningyou No.7 and NJ5412, from germplasm bank in Nanjing Agricultural University, were used for evaluation.

Essential oil

500 grams of fresh plants were harvested from jiangpu experiment field about 25 days before maturation, washed, cut into small pieces for volatile extraction. After water distillation in Clevenger apparatus along with 1200ml pure water for 6h, essential oil were extracted by aether, then the extracts were dried in vacuum to eliminate aether. Further drying of the oils were by anhydrous sodium sulphate for overnight and stored at -20°C for determination. Samples diluted with methylformate were injected into GC/MS for volatile molecule recognition.

Instrument analysis

The analysis were carried out with a Finnigan TRACE GC gas chromatograph coupled with a Finnigan TRACE DSQ mass spectrometer equipped with a DB-5 fused silica capillary column(30m × 0.25mm ID × 0.25μm film thickness).The oven temperature programmed from 60°C to 180°C at 6°C,then from 180°C to 220°C at 2°C,further from 220°C to 260°C at 8°C(30min isothermal). Carrier gas was high purity helium(flow rate 1ml/min).Injector temperature was 280°C and the split ratio was 1:30.The transfer-line temperature was 250°C.The mass spectra was acquired with a source temperature of 200°C under a 70eV ionization potential. Full-scan analyses were performed in the mass rang 30-400m/z.

Data was evaluated by Xcalibur 1.3 system software. Identification of the compounds was done by comparing the retention times and by the use of mass spectra database search (NIST MS search 2.0) and retention indices from accessible scientific literature as well as comparison of mass spectra from relevant literature.

Results and discussion

Volatile profiles identified by GC-MS (Fig.1), were quantitatively and qualitatively different among the varieties in *B.*

napus (table 1). The four varieties (Tapidor, Heza No.7, Ningyou No.7 and NJ5412) contained 55, 45, 51, and 44 kinds of compounds, respectively, accounting for 89.03%, 93%, 88.67% and 85.14% in their total volatiles, respectively. To sum up, there were a total of 107 different compounds identified, which may be classified as hydrocarbon, acid, aldehyde, ketone, alcohol, ester, isothiocyanate (ITC), nitrile (CN), sulfide and others. 15 compounds were common to four varieties, including high-content phytol and oleic acid, also including some flavor compounds such as β-damascenone, β-ionone and myristic acid,etc.

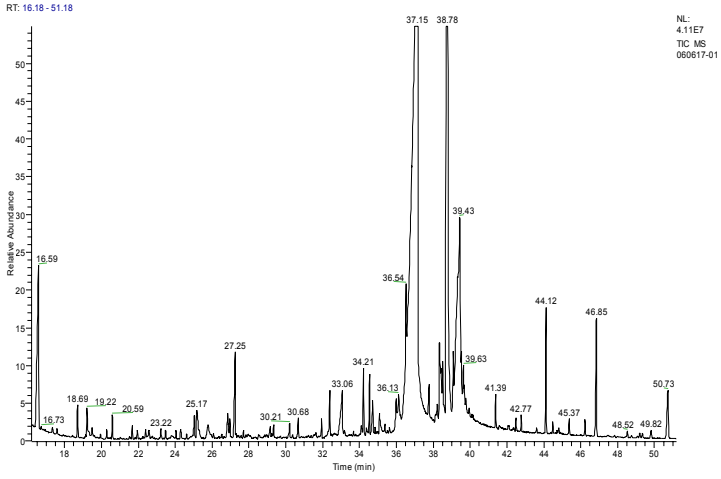

Fig.1 Typical chromatographic profile of the volatile fraction isolated from variety Tapidor

Acid fraction accounted for 63.98%, 73.97%, 68.91% and 62.52% in variety volatiles of Tapidor, Heza No.7, Ningyou No.7 and NJ5412, respectively. Acids with 18-carbon were main parts in acid fraction of volatiles, including hexadecanoic acid, oleic acid, linoleic acid, but for different varieties, main components and constitutions were different. Main parts were hexadecanoic acid (30.6%) and oleic acid (29.1%) for Tapidor, n-tridecanoic acid (25.6%) and oleic acid (40.9%) for Heza No.7, n-tridecanoic (32.1%) acid and linoleic acid (18.5%) for Ningyou No.7, n-tridecanoic acid (10.8%) and oleic acid (47%) for NJ5412. This phenomena that fatty acid may be high content in one variety volatile but low in another, may imply that different varieties have different metabolism mechanism or defense system. In addition, a interesting result may be that odd-numbered fatty acids unusual in plants, of high-content in three varieties of *B. napus*, were discovered for the first time.

Relatively nonreactive GLS in *B. napus* are easy to be hydrolyzed by myrosinase and converted to unstable aglycones. These intermediates then undergo a spontaneous rearrangement to produce a variety of toxic metabolites such as isothiocyanates (ITC), nitriles (CN), and thiocyanates [6]. By GC-MS technique that can be used as a way to volatile molecular identification, decomposed products like volatile ITCs and CNs can be identified. Present reports revealed 7 ITC, and 1 CN. From literatures, among the ITCs in this research, 3-butenyl ITC and 2-phenylethyl ITC were known to be involved in plant-insect communication [7]. GLS of ITC with cyclopentane side-chain structure was discovered firstly in *B. napus*, other GLS were in accordance with earlier reported in *brassicaceae* [8].

Agreeing with known information of varieties, this results revealed that GLS believed to be involved in plant-insect interactions as antibiotic effectors [9] and as antinutritional compounds to animals [10], was quantitatively and qualitatively different between double-high rapeseed and canola varieties, and for Ningyou No.7, containing 7 ITC and 1 CN implied its better stress tolerance. Except ITC, other 6 kinds of sulfur-containing volatiles characteristic of flavour and odour, were confirmed in our study. These sulfur-containing materials were also observed in earlier reports on biosynthetic induction experiments of sulfides regarded as effectors of insect herbivory [11].

Cis-3-hexenol as an insect attractant is the precursor for the straight-chain esters [12]. There was a remarkable increase in catches of the boll weevil when traps were baited with cis-3-hexenol combining with the boll weevil aggregation pheromone [13]. In addition, field baits of cis-3-hexenol and the pheromone enhanced the number of female *Plutella xylostella* in *B. oleracea subsp. capitata* caught in traps several fold over those baited with natural attractant alone [14]. Comparing varieties, we found that NJ5412 did not have cis-3-hexenol, but other three varieties had it with high content. This was a fact noteworthy, revealed that mechanism why canola line NJ5412 usually does not have insect damage.

In addition, other sense compounds such as thujopsene, safranal, β -linalol and 4-terpineol, were also identified, which are antibacterial molecules. Many other materials listed in table 1, are not discussed here. In total, different varieties had different volatile profiles that probably are basis of genotype selection.

Acknowledgements

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Table 1.The Relative contents and classes of compounds from Tapidor (Td), Heza No.7 (Hz), Ningyou No.7 (Ny) and NJ5412 (NJ)

Compounds	Td	Hz	Ny	NJ	Compounds	Td	Hz	Ny	NJ
Hydrocarbon					trans-Geranylacetone	0.18	0.05	0.06	0.13
cis-3-Nonene	-	-	-	0.75	2-Tridecanone	-	-	0.03	-
n-Dodecane	0.02	-	-	-	2-Hexadecanone	0.46	-	-	-
1,4,6-Trimethyl-1,2-dihydronaphthalene	-	-	-	0.38	Farnesyl acetone	0.37	-	-	-
2,5,8-Trimethyl-1,2-dihydronaphthalene	0.23	0.02	0.09	-	(2E)-1-(2,6,6-Trimethyl-1-Cyclohexen-1-yl)-2-Buten-1-one	0.18	-	-	0.11
Tridecane	0.02	-	-	-	Methyl 14-methylpentade canoate	0.16	-	-	-
Thujopsene	0.24	-	-	-	Hexahydeofamesyl acetone	0.36	0.12	0.42	0.22
2,6,10-Trimethyltetradecae	0.24	0.36	1	-	Methylheptadecyl ketone	-	0.34	0.42	0.3
Nonadecane	-	1.46	0.43	-	15-Nonacosanone	1.09	-	-	-
Heneicosame	5.58	-	-	-	**Alcohol**				
Heptacosane	-	0.45	0.42	1.53	cis-3-Hexenol	2.05	1.41	1.04	-
Acid					trans-2-Methylcyclopentanol	0.28	-	-	-
Hexadecanoic acid	30.6	0.69	7.63	0.59	cis-5-Octen-1-ol	-	-	-	0.59
Oleic acid	29.1	40.9	1.91	47	β-Linalol	-	-	0.01	-
n-Tridecanoic acid	-	25.6	32.1	10.8	4-Terpineol	0.03	-	0.04	-
Linoleic acid	0.75	1.25	18.5	0.4	β-Citrylideneethanol	-	0.12	-	-
d-(+)-Glyceric acid	0.02	-	-	-	1-Tridecanol	0.18		0.73	
Glutaconic acid	-	-	0.07	-	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl) but-2-en-1-ol	-	0.1	0.13	-
Decanoic acid	-	-	-	0.24	Phenol, 2,5-di-tert-butyl-	-	0.1	-	-
Lauric acid	-	0.52	0.48	0.56	Farnesol	-	0.19	-	0.56
Myristic acid	1.54	2.83	3.29	1.69	(2Z, 5Z) -2, 5-Pentadecadien-1-ol	-	-	-	0.05
(Z) -9-Tetradecenal	-	-	-	0.29	Hexa-hydro-farnesol	0.12	0.05	0.12	0.11
Pentadecylic acid	-	0.57	1.42	-	13-Heptadecyn-1-ol	-	-	0.21	-
9-Hexadecenoic acid	0.4	0.35	-	-	2-Methylhexadecan-1-ol	0.02	-	-	-
7-Methyl-Z-tetradecen-1-ol acetate	0.12	0.28	-	-	1,2-Methyl-E,E-2,13-octadecadien-1-ol	0.24	0.17	-	-
Z-10-Methyl-11-tetradecen-1-ol propionate	-	-	1.31	-	Phytol	5.43	8.93	7.42	4.94
α-Linolenic acid	0.12	-	2.03	-	**Ester**				
18-Nonadecenoic acid	-	-	-	0.89	Isovalericacid cis-3-hexenyl ester	0.05	-	-	-
cis-8,11,14-Eicosatrienoic acid	1.25	0.35	0.07	-	cis-3-Hexenyl benzoate	0.05	-	-	-
Z-14-Octadecen-1-olacetae	-	0.56	-	-	n-Hexyl salicylate	0.1	-	-	-
Aldehyde					Acetic acid, phenyl-, 3-hexenyl ester	0.03	-	-	-
Safranal	0.06	-	-	-	Phthalic, diisobutyl ester	0.63	-	-	-
β-Cyclocitral	0.12	0.05	0.12	-	Methyl (9E, 12E) 9,12-hexadecadienoate	-	-	-	0.19
2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	0.07	-	0.03	-	Octyl-10-undecenoate	-	0.45	-	-
(4E) -2-Methyl-4-octenal	-	-	-	0.05	Phthalic scid,butyl ctyl ester	-	0.49	0.86	0.13
n-Nonaldehyde	0.1	0.13	0.09	0.51	**Isothiocyanate(ITC)**				
(E) -2-Nonenal	-	-	-	0.08	3-Butenyl ITC	-	0.14	0.46	-
n-Decadehyde	0.13	0.09	0.07	0.11	2-Phenethylester ITC	0.33	1.46	1.21	0.48
(E,E)-2,4-Decadienal	-	-	0.03	0.24	Cyclopentane ITC	-	0.14	0.46	0.24
trans-2-Decenal	-	-	-	1.88	Butyl ITC	-	-	0.07	-
n-Undecanal	0.13	-	0.09	-	4-Methylpentyl ITC	-	0.02	0.09	-
2-Undecenal	-	-	-	0.33	Hexyl ITC	-	-	0.06	-
Dodecanal	-	-	0.15	-	n-Heptyl ITC	0.03	0.05	0.07	-
Tridecylic aldehyde	1.78	-	-	-	Nitrile(CN)				
Cinnamaldehyde,β-hexyl	-	-	0.03	-	3-Phenylpropionitrile	-	0.02	0.03	-
Myristaldehyde	1.27	0.9	2.44	0.94	**Sulfide**				
(11E)-11,13-Tetradecadienal	-	-	-	0.08	Trisulfide,dimethyl	0.06	0.1	0.06	1.45
(Z) -7-Tetradecenal	-	0.04	-	-	Dimethyl tetrasulphide	-	-	-	0.24
(Z)-7-Hexadecenal	-	-	-	0.35	Methyl methylthiomethyl disulfide	0.05	-	-	-
(3E)-13-Octadecenal	0.93	-	-	-	Methane,(methylsufinyl)methylthio-	-	-	-	0.03
14-Octadecenal	0.13	-	-	-	Sulfide isopentyl methyl	-	-	0.02	-
E-11-Octadecenal	-	-	-	0.5	Sulfide,heptylmethyl	-	0.03	-	-
Ketone					**Others**				
4,5-Dihydro-2 (1H)-pentalenone	0.15	-	-	-	D-Mannose	-	0.02	0.02	-
(2E) -2-Nonen-4-one	-	-	-	0.11	1-Methoxy-1H-indole	-	-	0.04	-
2-Methyl-1-none-3-one	0.05	-	-	-	3-(1-Cyclopentenyl) furan	0.02	-	-	-
β-Damascenone	0.19	0.12	0.18	0.13	Guaia-1 (5),7(11)-diene	-	-	-	0.48
β-Ionone	0.9	0.45	0.51	0.11	8β,13β-Kaur-16-ene	-	0.46	-	4.29
β-Damascone	0.21	-	-	-					

References

Dudareva, N., Pichersky, E. and Gershenzon, J. 2004. Biochemistry of plant volatiles. Plant Physiol, **135**:1893–1902.

Pichersky, E. and Gershenzon, J. 2002. The formation and function of plant volatiles: Perfumes for pollinator attraction and defense.Current opinion in plant biology, 5:237-243.

Kessler, A. and Baldwin, I.T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science, **291**: 2141–2144.

Engelberth, J., Alborn, H.T., Schmelz, E.A. and Tumlinson, J.H. 2004. Airborne signals prime plants against insect herbivore attack. Proceedings of the National Academy of Sciences, **101**:1781–1785.

Bridges, M., Jones, A.M., Bones, A.M., Hodgson, C., Cole, R., Bartlet, E., Wallsgrove, R.M., Karapapa, V.K., Watts, N. and Rossiter, J.T. 2002. Spatial organization of the glucosinolate–myrosinase system in Brassica specialist aphids is similar to that of the host plant. Proceedings of the Royal Society of London B, **269**: 187–191.

Rora, E.A.S., Heaney, R.K., Fenwick, G.R. and Portas, C.A.M. 1997. Glucosinolates in crop plants. Horticultural Reviews, 19: 99-215.

Wittstock, U., Kliebenstein, D.J., Lambrix, V., Reichelt, M. and Gershenzon, J. 2003. Glucosinolate hydrolysis and its impact on generalist and specialist insect herbivores. In: Romeo, J. (Ed.),From Ethnobotany to Molecular Ecology, Recent Advances in Phytochemistry, Elsevier, Oxford, 37.pp. 101–125.

Fahey, J. W., Zalcmann, A. T. and Talalay, P. 2001. The chemical diversity and distribution of **glucosinolates** and isothiocyanates among plants. Phytochemistry, 56:5-51.

Rask, L., Andreasson, E. B., Eriksson, S., Pontoppidan, B. and Meijer, J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Molecular Biology, 42: 93-113.

Griffiths,D.W., Birch,A.N.E., Hillman,J.R. 1998. Antinutritinal compounds in the Brassicaceae: analyses, biosynthesis, chemistry and dictary effects. Journal of Horticultural Science and Biotechnology, 73:1-18.

Van Poecke, R.M.P., Posthumus, M.A. and Dicke, M. 2001. Herbivore-induced volatile production by Arabidopsis thaliana leads to attraction of the parasitoid Cotesiarubecula: chemical,behavioral, and gene-expression analysis. Journal of Chemical Ecology, 27:1911–1928.

Rowan, D. D., Allen, J. M., Fielder, S., and Hunt, M. B. 1999.Biosynthesis of straight-chain ester volatiles in red delicious and granny smith apples using deuterium-labeled precursors. Journal of Agricultural and Food Chemistry, 47, 2553–2562.

Dickens,J.C. 1989. Green leaf volatiles enhance aggregation pheromone of the boll weevil Anthonomus grandis. Entomologia experimentalis et applicata, 52: 191-203.

Reddy,G.V.P. and Guerrero,A. 2000. Behavioral responses of the diamondback moth, Plutella xyclostella, to green leaf volatiles of Brassica oleracea subsp. Journal of Agricultural and Food Chemistry, 48: 6025-6029.

Influence of pH and type of myrosinase complex on the products obtained in the myrosinase catalysed hydrolysis of glucosinolates —a MECC study

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Abstract

Environmental conditions, e.g. pH and the presence of Fe^{2+} are well known factors that influence the product profile of the myrosinase catalysed hydrolysis of glucosinolates. Depending on the plant genera, the species and tissue of origin myrosinase isoenzymes (thioglucohydrolase EC 3.2.1.147) have different characteristics in terms of MW, subunit composition and pI. However, the influence of these parameters on the outcome of glucosinolate hydrolysis has not been traditionally studied, which hinders the full exploitation of the catalytic potential of these enzymes. In the present experiments the effect of myrosinase type on the products obtained in the hydrolysis of glucosibarin was studied by MECC using two *B. carinata* myrosinase preparations differing on their affinity to the Con A material, Con A 1 (first eluting fractions) and Con A 2 (last eluting fractions). At pH 3 Con A 1 isoenzymes were more active than Con A 2 isoenzymes. At pH 5 and 6.5 Con A 1 isoenzymes produced oxazolidine-2-thione to a higher extent than Con A 2 isoenzymes. The production of nitriles by Con A 1 isoenzymes was not influenced by pH and at pH 5 and 6.5 the amount of nitrile produced by Con A 1 isoenzymes was lower than that produced by Con A 2 isoenzymes. Formation of nitriles requires the presence of two redox equivalents which leads to the release of the sulphur atom from the aglucone. Isothiocyanates and nitriles differ in their bioactivity towards different targets; therefore the possibility for directing the glucosinolate hydrolysis towards the desired compound in a particular situation is of great relevance.

Introduction

Glucosinolates are amino acid derived allelochemicals present in all plants of the order Capparales. They co-exist with myrosinase isoenzymes (EC 3.2.1.147), which are oligomeric glycoproteins that catalyze the hydrolysis of the β-D-thioglucopyranoside bond. The aglucone released in the hydrolysis of the glucosinolate further rearranges to a variety of products depending on the parent glucosinolate and the environmental conditions (Sørensen, 1990; Bjergegaard et al., 1994). The great variety of physiological effects of the different glucosinolate derived compounds makes it very relevant to study the factors controlling glucosinolate hydrolysis and the conditions under which the different transformation products are produced. It is well established that pH and the presence of certain cofactors have an influence on the compounds formed, e. g. at neutral pH aliphatic glucosinolates generally yield isothiocyanates, while at acidic pH or in the presence of Fe^{2+} the formation of nitriles is favoured (Sørensen, 1990; Bjergegaard et al., 1994). Myrosinase isoenzymes have different molecular weight, pI and subunit composition (Bellostas et al., 2003), however, little is known about whether these parameters influence the type and quantity of compounds formed upon glucosinolate hydrolysis. In a previous study we have developed a method for the on-line monitoring of the myrosinase hydrolysis of glucosinolates which allows for the simultaneous detection of the degradation compounds (Bellostas et al., 2006). With the use of this method in the present experiments we have studied the influence of the type of myrosinase isoenzymes on the outcome of the hydrolysis of glucosibarin at three pH values (3, 5 and 6.5).

Materials and methods

Glucosinolates and myrosinases

The intact glucosinolates were from the laboratory collection (Sørensen, 1990, Sørensen, 2001). Myrosinase isoenzymes were obtained from *B. carinata* cv. BRK-147-A by the method used in our laboratories (Bellostas et al., 2003). After affinity chromatography by Con A, two pools were made (Figure 1): Con A 1 (first six fractions from the Con A column) and Con A 2 (seven last fractions from the Con A column).

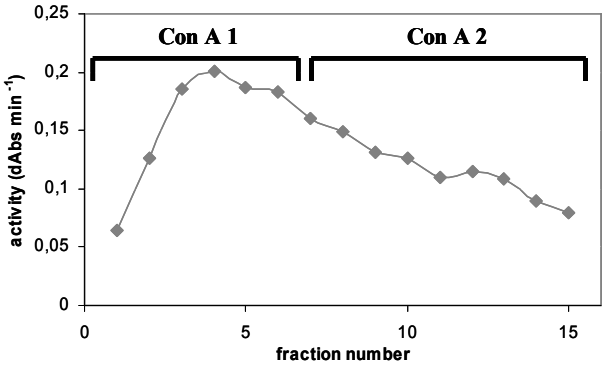

Figure 1. Activity (dAbs min^{-1} at 227 nm) of the fifteen different fractions eluted from the Con A affinity column.

CE instrumentation, buffer and procedure for the in-vial reaction

The CE instrumentation, buffer composition and procedure used for the in-vial reaction have been described elsewhere (Bellostas et al., 2006).

Results and discussion

Profile of degradation products depending on pH and myrosinase type

As previously described (Sørensen, 1990) pH has a great influence on myrosinase activity and therefore the rate of degradation of glucosibarin by both groups of isoenzymes decreased when pH was decreased from 6.5 to 3. Differences between the two groups of isoenzymes could still be observed, and whereas no activity of Con A 2 isoenzymes was detected at pH 3, Con A 1 isoenzymes still degraded glucosibarin at this low pH. At pH 6.5 and 5 Con A 1 isoenzymes produced OZT almost in a linear fashion with time, whereas Con A 2 isoenzymes seemed to produce OZT only in the first minutes of the reaction. At pH 3 only low amounts of OZT were produced by both isoenzyme groups (Figure 2 above). At pH 5 and 6.5 Con A 2 isoenzymes produced the nitrile in a higher concentration than Con A 1 isoenzymes, the highest amount of nitrile being produced by Con A 2 isoenzymes at pH 5. At pH 3 Con A 2 isoenzymes produced the nitrile only up to a plateau, probably due to a low activity of the isoenzymes at this pH. Although the final amount of nitrile produced at the different pHs by Con A 1 isoenzymes was very similar, this compound was produced at different moments in time: the lower the pH the earlier the nitrile was produced (Figure 2 below).

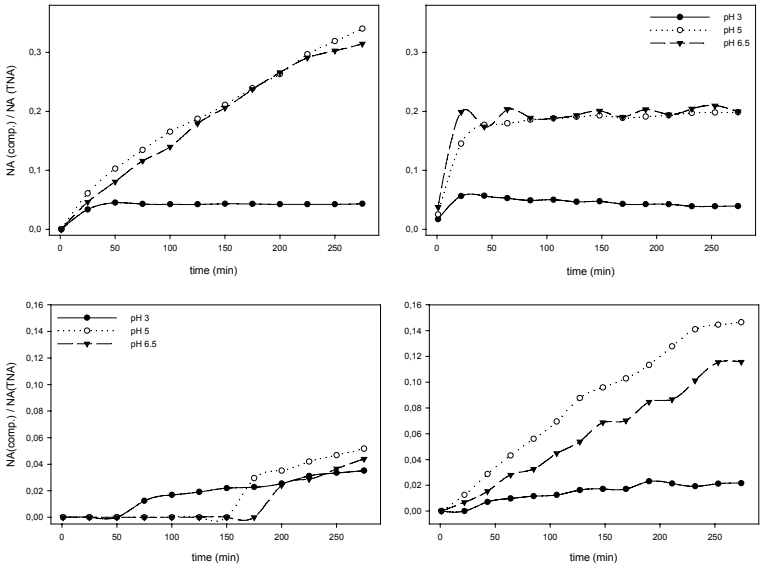

Figure 2. Production of the OZT (above) and the nitrile (below) at three different pH by Con A 1 (left) and Con A 2 (right) isoenzymes. Values are presented as relative normalized area of the compound with respect to TNA.

Physico-chemical properties of myrosinase isoenzymes and their relation to the profile of glucosinolate hydrolysis products

Con A 1 and Con A 2 isoenzymes groups are retained in the affinity chromatography column to different extents, therefore it is likely that differences in the glycosylation level of the protein subunits play a role in the activity of the isoenzymes. Figure 3 shows the SDS-PAGE gels of *B. carinata* Con A 1 and Con A 2 isoenzymes groups before and after G-200 gel filtration (Bellostas et al., 2003). Although showing very similar profiles, a number of subunits present in one group of isoenzymes seem to be absent in the other one and vice versa (see arrows). Whether a different protein profile of the

myrosinase complexes may have an influence on the myrosinase catalysed glucosinolate hydrolysis to the extent of changing the product profile has not been described, although the presence of some proteins in the myrosinase complexes leads to the production of special compounds, such as epithionitriles (de Torres Zabala et al., 2006).

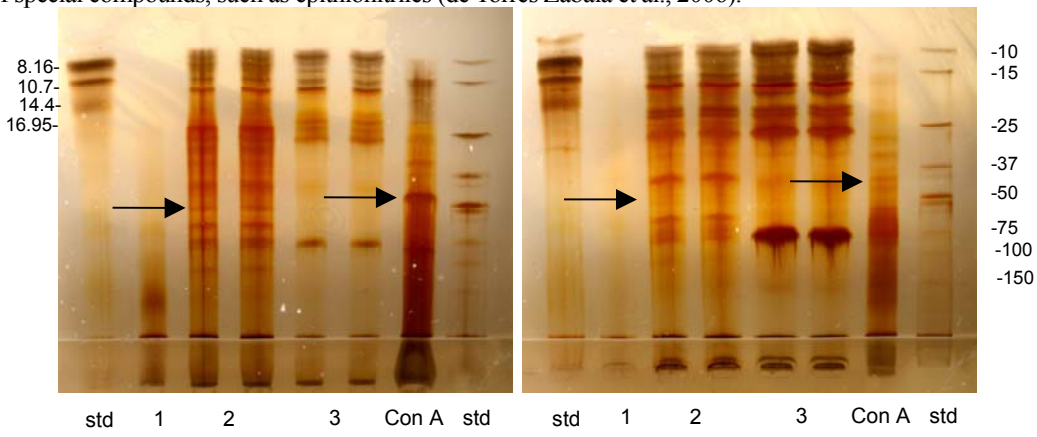

Figure 3. SDS-PAGE of *B. carinata* isoenzymes after G-200 gel filtration. Left: Con A 1 isoenzymes. Right: Con A 2 isoenzymes. Legend: std (standard), 1, 2 and 3 (first, second and third peaks in G-200), Con A (Con A pool before G-200 gel filtration).

Conclusions

The MECC method allowed for the observation of different degradation patterns of the two groups of isoenzymes that would not have been observed with a standard spectrophotometric assay, which confirms the validity of the method developed. The different pools of myrosinase isoenzymes obtained after Con A affinity chromatography produced different proportions of OZT and nitrile at the three pH studied. This opens the door for further research, as the use of different myrosinase isoenzymes may allow directing the hydrolysis of glucosinolates towards the wanted compounds.

Acknowledgement

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References

Bellostas N, Jørgensen ALW, Lundin NVF, Petersen IL, Sørensen H, Sørensen JC, Sørensen R, Tidmand KD. 2003. Comparison of physico-chemical properties of myrosinase isoenzymes in seeds of *Brassica* species of the U triangle, in *Proceedings of the GCIRC 11th International Rapeseed Congress*, Copenhagen, pp. 720-723.

Bellostas, N., Sørensen, J. C., Sørensen, H. 2006. Micellar electrokinetic capillary chromatography – Synchronous monitoring of substrate and products in the myrosinase catalysed hydrolysis of glucosinolates. J. Chromatography A, 1130, 246-252.

Bjergegaard C, Li PW, Michaelsen S, Møller P, Otte J, Sørensen H. 1994. Glucosinolates and their transformation products - compounds with a broad biological activity. In Kozlowska, H., Fornal, J., and Zdunczyk, Z. (Eds.). Bioactive substances in food of plant origin. Polish Academy of Sciences, Poland, 1: 1-15

De Torres Zabala, M., Grant, M., Bones, A. M., Bennett, R., Lim, Y. S., Kissen, R., Rossiter, J. T. 2005. Characterisation of recombinant epithiospecifier protein and its over-expression in *Arabidopsis thaliana*. Phytochemistry, 66, 859-867.

Sørensen, H. 1990. Glucosinolates: Structure – Properties – Function. In *Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology;* Shahidi, F. Ed.; Van Nostrand Reinhold Publishers: New York, pp. 149-172.

Sørensen J. C. 2001. Biorefined oilseed products – high quality products with added value. PhD Thesis. Chemistry Department, KVL, Denmark.

QUALITY, NUTRITION AND PROCESSING

Processing Technology

Research survey and prospect on comprehensive processing of rapeseed

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Abstract

Rape is the most preponderant oil plants crop in China, which is planted widely. Its yield reached 12 million tons in 2005, ranked first in the world. Besides oil, rapeseed contains many other components, such as protein, glucosinolate, polyphenol, phytic acid, polysaccharide, cellulose, sterol, vitamine E and so on. Most of them have great utilized values, which reveal a fine prospect for the comprehensive utilization of rapeseed. Therefore, the research and development of comprehensive processing techniques of rapeseed is all along a hotspot around the world. In this paper, we introduced the research on rapeseed comprehensive processing in our laboratory, and display the vision of the developing direction in the field.

Key words:rapeseed,comprehensive processing, Research Survey,prospect

Rape is the most preponderant oil plants crop in China, which is planted widely. Its yield reached 12 million tons in 2005, ranked first in the world. Besides oil, rapeseed contains many other components, such as protein, glucosinolate, polyphenol, phytic acid, polysaccharide, cellulose, sterol, vitamine E and so on. Most of them have great utilized values, which reveal a fine prospect for the comprehensive utilization of rapeseed. Therefore, the research and development of comprehensive processing techniques of rapeseed is all along a hotspot around the world. In this paper, we introduced the research on rapeseed comprehensive processing in our laboratory, and display the vision of the developing direction in the field.

1 Basic research of rapeseed

1.1 Rapeseed protein

1.1.1 Classification of rapeseed protein isolate

Albumins and globulins were found to be the predominant proteins in Hua-Za 3 meal, comprising 36.8% and 31.6% of total proteins. While glutelins and prolamins were 29.1% and 2.5%,respectively. The molecular weight of fractionation was shown by SDS-PAGE and the structure of albumins is simple. Amino acid profile of the isolates indicated that the essential amino acid of each protein fraction nearly reach to 50%. Albumins have better function, including water absorption, oil absorption, emulsifying activity and emulsion stability. In addition, the albumin maintains their native capability because of low-temperature pressing, So albumin can be widely applied in the food and beverage industry.

1.1.2 Preparation of rapeseed peptides

Double-enzyme can not only increase the DH,but remove the bitter of RSP. In our experiment, Rapeseed Albumin was hydrolyzed with sequentially alcalase and flavourzyme. Degree (DH) were used as response values in analysis of response surface regression (RSREG), considering the mouth-sense, the optimum conditions of alcalase enzymatic hydrolysis have been determined by mono-factor analysis and response surface methodology as follows, pH :8.0, hydrolyzing temperature: 50.1°C,enzyme concentration: 0.38AU per gram of substrate, concentration of substrate:4.87%. Flavourzyme is used by step for 2h after reacted 1h with alcalase, the degree of hydrolysis of rapeseed albumin can go up to 28%.

Hydrolysates were clarified by filtration to remove insoluble substrate fragments. and the filtrate was lyophilized and freeze-dried for further use. The hydrolysates (RSP-R) was graded by Sephadex G-25 column with distilled water as eluant,fractions were pooled into three major groups(RSP-1, RSP-2 and RSP-3).Protein content in RSP-1,RSP-2 and RSP-3 were 71.76%, 79.23% and 86.14%;crude polysaccharides were 22.64%, 13.88% and 5.18%. Molecular weight of each grade were bigger than 5000,1052 and 563 Dal. Contents of amino acids determined by HPLC in three grades differed from each other.

1.1.3 Antioxidant activities of rapeseed peptides

After injecting 50, 100 mg/kg·d 15d for 15 d, MDA levels in serum in the RSP group were obviously lower than that of the control ($P < 0.05$) ;So RSP could improve the antioxidant capacity in vivo. The results in vitro showed that RSP had higher reductive activity and can scavenge hydroxyl radicals.

RSP had powerful inhibiting activity on active oxygen within certain concentration. As for malondialdehyde(MDA) formation and H_2O_2-induced of mice liver,RSP-R and RSP-1 had better inhibiting effects than RSP-2 and RSP-3,exhibiting dosage-depended. but little inhibiting effect on MDA induced by Fe^{2+}.There were not much influence of RSP on hemolysis of mice red blood cell. In a word, antioxidant activities of RSP-R and RSP-1 were better than others.

1.1.4 RSP can inhibit the growth of S_{180} tumor,the mechanism may have something to do with its antioxidant activities and increasing organize immune capability,RSP may induce the apoptosis of Hela cells as well.

RSP-R 100,150 mg/kg·d could inhibit the growth of S_{180} and increase thymus weight of S_{180}-bearing mice. Compared with cyclophosphamid, it did not decrease the immune viscera weight and interference the growth of S_{180}-bearing mice. RSP-R had little influence on macrophage phagocytosis and delayed-type hypersensitivity except high dose.But RSP-R could effectively improve the content of serum hemolysin IgM evidently.In addition, MDA levels in serum in the RSP group were obviously lower than that of the control, SOD activities in serum were significantly increased.The results showed that antioxidant activities and increasing organize immune capability of RSP were the most mechanism of its inhibiting tumor effect.

This dissertation demonstrate that the three grades of RSP can induce the apoptosis of Hela cells.RSP display evidently growth inhibitory effect in a dose-and time-dependant manner against Hela cells by MTT assay.

Fluorescence and electron microscopy assay illuminated that RSP can induce apopt- osis of Hela cells, which is dosage-dependent. So the inducing effect on the apoptosis of Hela cells may be one of anti-tumor effect mechanisms of RSP.

The attainting effect of RSP on Hela DNA can be assayed by single cell gel electrophoresis(SCGE),gel electrophoresis and TUNEL assay. In the distributions figure of cell cycle, S stage cells apparently increased through flow cytometry fluorescence. So RSP-2 can arrest Hela cell growth in S phrase. In addition, the results of RT-PCR showed that RSP-2 can decrease the expression of Bcl-2 gene.

1.1.5 This dissertation demonstrated the inhibiting effect of RSP on angiotension I-converting enzyme(ACE) activity of SHR. (*in Vitro*)

Rapeseed peptide with inhibiting effect on ACE of SHR can be gotten by hydrolysis rapeseed albumin. The peptide have the strongest inhibiting effect after hydrolyzed for 1h, it can reaches 42.68%. Hydrolysis with double-enzyme can improve effectively the inhibiting effect of RSP on ACE activity. The inhibiting activity was 69.13% when hydrolysis for 2hrs with alcalase and flavourzyme.

RSP-R and its three grades (isolated through Sephadex G-25) exhibited ACE inhibitory activity. Among which, RSP-3 showed the highest inhibition. When the RSP-3 concentration was augmented to 1mg/ml,the inhibition of ACE activity could nearly achieve to 96%.While RSP-2,RSP and RSP-1 were 78.43%, 69.13% and 60.92%, respectively, which illuminated RSP can be as a higher inhibition factor of ACE activity, All the results will extend the utilizing fields of RSP.

1.2 The rapeseed polyphenol

1.2.1 Structure of rapeseed polyphenol

The rapeseed polyphenol was purified by macroreticular resin, and separated by Sephadex LH-20, fractions were collected and marked as RSPP-0, RSPP-1, RSPP-2, RSPP-3, RSPP-4, RSPP-5, RSPP-6, RSPP-7. There were peaks near 330nm and 280nm in UV-vis spectra of fractions mentioned above. Peaks near 330nm denoted phenolic acids and peaks in 270—282.6nm denoted flavones, flavonoles or tannin, or hydroxylcinnamic acid. The IR spectra of fractions mentioned above were similar to the spectra of catechin and contained the structure information of phenyl and hydroxyl. RSPP could be separate well by gradient elution.

There were pseudomolecular ions (negative ion mode) which were equal to the cyaniding, and gallate + Na^+, and dicaffeoylquinic, and procyanidin dimmer, 1,2-disinapoly-2-feruloyldiglucoside, procyanidin trimer, 1, 2, 2’ — trisinapoldiglucoside in m/z.

There were 5 substance in HPLC-DAD-ESI-MS/MS spectra(negative ion mode) of RSPP. Substance 2 was correspond to 1,2-disinapoly-2-feruloyldiglucoside in pseudomolecular and fragment ions, substance 5 was correspond to kaempferol 3-sinapoyldiglucose. Substance 3 was the isomeric compound of substance 2 and 4 was 5. The structure of substance 1 could not be identified and its m/z of pseudomolecular and fragment ions were 494.2 and 462.0, it was abundant in RSPP. Substance 2 and 5 were reported from rapeseed for the first time.

1.2.2 Bioactivities of rapeseed polyphenol

1.2.2.1 The anti-oxidative effects and mechanism of RSPPs

The effects of RSPPs (including RSPP and RSPP-1,RSPP-2, RSPP-3,RSPP-7)on anti-oxidative in some modified chemical systems, mice liver mitochondria, rat and mice red blood cell(RBC), mice serum and liver homogenate and mice in vivo were measured. The results showed that RSPPs were good deoxidant and could scavenge reactive oxygen species and inhibit lipoxygenase in some modified chemical systems. The capabilities of high deoxidating and inhibiting the enzymes linked with oxidation were revealed to be the mechanism of inhibiting oxidation of RSPPs. In vitro, RSPPs could inhibit the swelling of mice liver mitochondria and the auto-oxidation hemolysis of rat RBC and the hemolysis of mice RBC induced by H_2O_2 as well. RSPPs could also heighten the anti-oxidation capability of mice serum, and inhibit the formation of malondialdehyde(MDA) in mice liver mitochondria, rat RBC and mice liver homogenate. In vivo, there was a significant difference ($p<0.01$)between the controled group and the group *intra- peritoneal* injected with RSPP in MDA value of mice liver homogenate. All the above experiments indicated that RSPPs had evident anti-oxidation function in vivo.

1.2.2.2 Inhibiting Effects of RSPP on proliferation of Human Hepatocellular Carcinoma Cell Line SMMC-7221

RSPP at concentration of 25-400μg/mL displayed evidently growth inhibitory effects in a dose-and time-dependant manner against SMMC-7721 cells by MTT assay. Inhibiting rate of 400μg/mL RSPP for 3 days was 70.18%. The results of MTT assay could be authenticated by the results of proliferating cell nuclear antigen(PCNA) assay.

The results of morphological observation and alpha-fetoprotein(AFP) assay showed that RSPP could induce SMMC-7721 cells to differentiate into normal cells. According to the results of flow cytometer assay, RSPP could block SMMC-7721 cells in S-phase but could not induce SMMC-7721 cells apoptosis.

It was possible to infer 2 mechanisms for RSPP inhibiting SMMC-7721 cells from the results, one was that RSPP blocked SMMC-7721 cells in S-phase, another was that RSPP induced SMMC-7721 cells to differentiate into normal cells.

1.2.2.3 Effects of polyphenol from rapeseed on S_{180} tumor growth and organize immune capability in vivo

The tumor-bearing mice were gained by implanted S_{180} cells sustained in right front axillary. After *intra- peritoneal* injected samples 10d, the mice were executed. Tumor weight showed that RSPPs could inhibit tumor growth in vivo and the inhibiting rate of 50~200 mg/kg·d RSPP were between 30.23% and 44.19%, 50 mg/kg·d RSPP-1 and RSPP-3 were 39.53% and 32.56% respectively.

The tumor tissue were stained by HE, and pathology observation showed that the phenomena of condensation of the nucleus and phlegmonosis cells infiltration and degranulation of polymorphonuclear neutrophils appeared much more in RSPPs groups than in tumor control group, and the area of dead tumor cells in RSPP groups were bigger than the area in tumor control group. It was proved that RSPPs could inhibit tumor growth and increase organize immune capability.

The thymus index, spleen index, macrophage phagocytic rate and phagocytic index,delayed-type hypersensitivity,splenic antibody formation and serum hemolysin content of tumor-bearing mice could be increased significantly by RSPPs. RSPPs were beneficial to immunity of mice.

RSPPs could inhibit the activity of mice lactate dehydrogenase, heighten the activity of mice catalase, decrease the content of MDA in mice serum. The inhibiting tumor mechanism of RSPS may have something to do with its antioxidant activities and inhibition of lactate dehydrogenase

1.2.2.4 The anti-hyperglycemic activity of rapeseed polysacchrides in vivo

Diabetes mellitus mice were induced by alloxan and RSPP were administrated by *intra- peritoneal* injection for 12d. The serum glucose of mice showed that The serum level of blood glucose in diabetes mellitus mice decrease 17.99% and 24.14% in the 75 and 400mg/kg·d groups while remained no obvious change in the normal group injected 400mg/kg·d RSPP.

Compared with control group, the body increase and liver index and kidney index of the normal groups injected 400mg/kg·d RSPP remained no obvious change. RSPP at dose of 75 and 400mg/kg·d both could increase thymus index and spleen index significantly.

RSPP at dose of 400mg/kg·d could decrease MDA content in liver homogenate of normal mice, but have not effect on anti-ROS unit in serum. RSPP at dose of 75 and 400mg/kg·d both could decrease MDA content in liver homogenate and increase anti-ROS unit in serum of diabetes mellitus mice significantly.

The kidney and liver tissue were stained by HE, and pathology observation showed that PHF and RSPP could not prevent diabetes mellitus doing harm to kidney, but protect liver against diabetes mellitus.

1.3 Bioactivities of byproduct of extraction of rapeseed polysaccharides

1.3.1 The Anti-Oxidative Effect and Mechanism of rapeseed Polysaccharide (RSPS)

The anti-oxidative effect and mechanism of RSPS were studied. The deoxidation was measured by $K_3[Fe(CN)_6]$ and TCA system, the inhibition on lipoxygenase was detected by crude lipoxygenase**,**the content of malondialdehyde(MDA) and reactive oxygen species (ROS) were analysed by the reagent kits, the swelling of mice liver mitochondria were observed by the spectrophotometric method. The results showed that RSPS was a good reducer, the anti- ROS unit of 2.00 $mg \cdot ml^{-1}$ RSPS was 94.03,the inhibiting rate of 2.00 $mg \cdot ml^{-1}$ RSPS on lipoxygenase was 22.8% in some chemical modified systems**;** 2.00 $mg \cdot ml^{-1}$ RSPS made the radical-induced swelling of mice liver mitochondria be lower than the swelling of mice liver mitochondria without inducement; the inhibiting rate of 2.00 $mg \cdot ml^{-1}$ RSPS on radical-induced MDA formation are 30.3% in mice liver mitochondria, 54.7%(incubation) and 32.0%(incubation with Fe^{2+}) and 84.5%(incubation with H_2O_2) in liver homogenate respectively, the anti- ROS unit of mice serum added 10.00 $mg \cdot ml^{-1}$is 1340.13 *in vitro***;** *in vivo*, There was a significant difference(P<0.01) between the controlled group and the *intra- peritoneal* injected RSPP (400$mg \cdot kg^{-1}$ $bw \cdot d^{-1}$, 12d) group in MDA value in mice liver homogenate. All the results above showed that RSPS had anti-oxidation effect *in vitro* and *in vivo*, the capabilities of high deoxidation and inhibiting the enzymes associated with oxidation were revealed to be the possible mechanism of RSPS to inhibit oxidation.

1.3.2 Effects of rapeseed polysaccharides on S_{180} tumor growth and organize immune capability in vivo

The tumor-bearing mice were gained by implanted S_{180} cells sustained in right front axillary. After *intra- peritoneal* injected samples 10d, the mice were executed. Tumor weight showed that RSPS could inhibit tumor growth in vivo and the inhibiting rate of 50~200 mg/kg·d RSPS are between 20.66% and 34.71%.

The tumor tissue were stained by HE, and pathology observation showed that the phenomena of condensation of the nucleus and phlegmonosis cells infiltration and degranulation of polymorphonuclear neutrophils appeared much more in RSPS groups than in tumor control group, and the area of dead tumor cells in RSPS groups were bigger than the area in tumor control group. RSPS could inhibit tumor growth and increase organize immune capability.

The thymus index, spleen index, macrophage phagocytic rate and phagocytic index,delayed-type hypersensitivity,splenic antibody formation and serum hemolysin content of tumor-bearing mice could be increased significantly by RSPS and RSPS were beneficial to immunity of mice.

RSPS could inhibit the activity of mice lactate dehydrogenase, heighten the activity of mice catalase, decrease the content

of MDA in mice serum. The inhibiting tumor mechanism of RSPS may have something to do with its antioxidant activities and inhibition of lactate dehydrogenase.

1.3.3 The anti-hyperglycemic activity of rapeseed polysaccharides in vivo

Diabetes mellitus mice were induced by alloxan and RSPS were administrated by 12d *intra- peritoneal* injection. The serum glucose showed that The serum level of blood glucose in diabetes mellitus mice decreased 18.38% and 24.62% in the 75 and 400mg/kg·d groups while remained no obvious change in the normal group injected 400mg/kg·d RSPS.

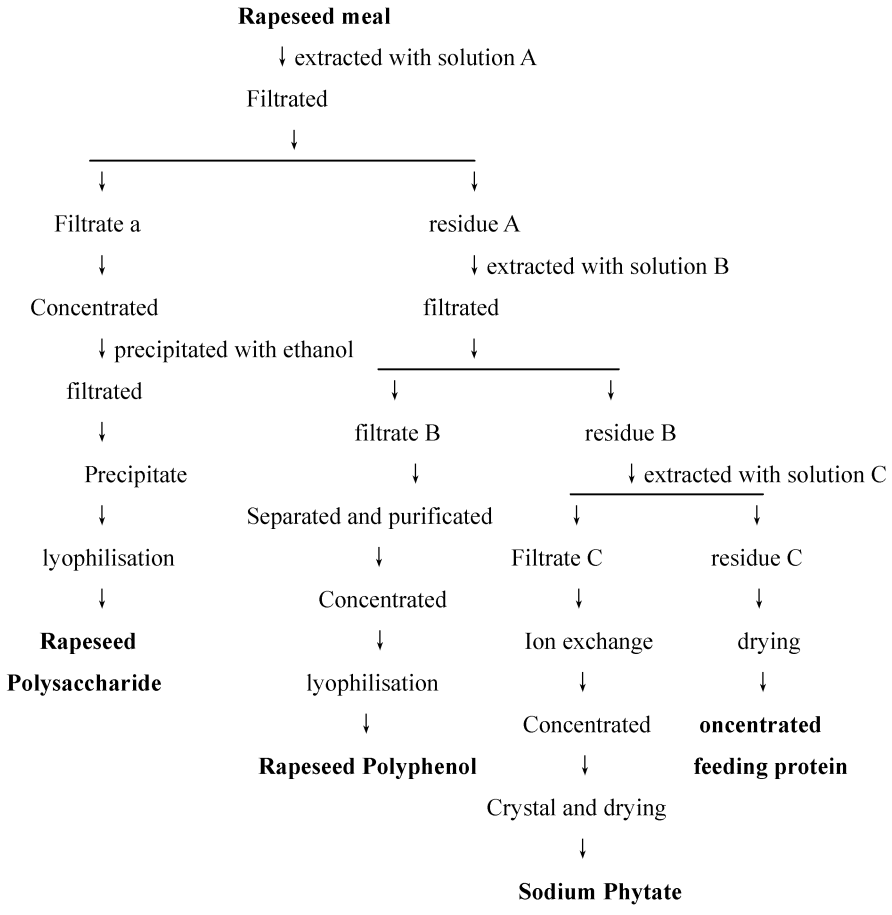
Compared with control group, the body increase and liver index and kidney index of the normal groups injected 400mg/kg·d RSPS remained no obvious change. RSPS on dose 75 and 400mg/kg·d both could increase thymus index and spleen index significantly.

RSPS on dose 400mg/kg·d could decrease MDA content in liver homogenate of normal mice, but have not effect on anti-ROS unit in serum. RSPS on dose 75 and 400mg/kg·d both could decrease MDA content in liver homogenate and increase anti-ROS unit in serum of diabetes mellitus mice significantly.

2. Studies on the process route of comprehensive utilization of rapeseed meal

2.1 Process route

We brought forward a process route of extracting polyphenol, polysaccharide and phytic acid from the rapeseed meal step by step as well as innoxious, high biological-performance concentrated protein, which was used to feed animals.



2.2 Preparation of rapeseeds polysaccharides (RSPS)

RSPS was extracted with water and then precipitated with ethanol, while the protein, the polyphenol and the phytic acid was also isolated respectively from the rapeseed cake and meal simultaneously. The optimized technical condition of isolating RSPS was studied through orthogonal test and the result was following: the rapeseeds cake and meal was marinated with 25 times of hot water at 100°C for 4 hours by solvent A. The yields of rapeseeds polysaccharides was 2.8% under this condition.

2.3 Extraction of polyphenol, phytic acid and protein in the rapeseed meal

The way of comprehensive utilization of polyphone, physic acid and protein in the rapeseed meal by extraction with solvents was studied; The extraction technology was optimized by fractional factorial design, central composite design and response surface analysis; mathematical models for predicting the extraction ratio of polyphone and physic acid were set up.

$$y_1=28.21260+0.29714x_1+0.03635x_3+0.06031x_4-0.39723x_1^2-0.43611x_3^2-0.21342x_4^2 -0.40250x_1x_3+0.01750x_1x_4+0.01500x_3x_4 \quad (1)$$

$$y_2=2.36842+0.15874x_1-0.11381x_3+0.12657x_4+0.11426x_1^2+0.38095x_3^2-0.13458x_4^2 +0.13463x_1x_3+0.19388x_1x_4+0.07588x_3x_4 \quad (2)$$

$$y_3=25.33261+0.20798x_1+0.61171x_3+0.16620x_4-0.05275x_1^2-0.80386x_3^2-0.31608x_4^2 +0.04000x_1x_3+0.60500x_1x_4+0.38000x_3x_4 \quad (3)$$

$$y_4=1.06082-0.05012x_1-0.01679x_3-0.00301x_4+0.13483x_1^2+0.01483x_3^2+0.02066x_4^2 +0.01538x_1x_3-0.04063x_1x_4-0.04313x_3x_4 \quad (4)$$

y_1, y_2 are the concentration of polyphenol and phytic acid in the extraction liquid of polyphenol respectively.y_3, y_4 are the concentration of phytic acid and polyphenol in the extraction liquid of phytic acid respectively. The four equations are anastomosed to the experimental data.

The results showed that 6 ml/g rapeseed cake, 65%(v/v) solvent B, 1.3g/100ml assistant substance, 51°C, 20min and extracting 3 time for obtaining polyphenol, 1%(v/v) solvent C, 20~30°C and extracting 2h for obtaining phytic acid were the optimized conditions. The extraction ratio of polyphenol about 2.82%, phytic acid 2.34% and protein content 70.2% respectively. The poisonous glucosinolate and anti-nutritious polyphenol and phytic acid in the protein were 0.76umol/g, 1.33mg/g, and 3.01% respectively, which declined 96.6%, 95.9%, and 38.5% accordingly.

2.3.1 Separation and purification rapeseed polyphenol

Experiments of 8 kinds of macroreticular resin adsorbing and desorbing rapeseed polyphenol showed that special 1 was the most appropriate resin in all for the preliminary purification of polyphenol, and the adsorbing capacities of resin could be utilized fully by arranging column in series. In column of special 1,impurity in rapeseed polyphenol could be removed well, and these condition was best for purifying polyphenol in mass production: 74%(v/v) acidic ethanol (containing 0.5%(v/v) 0.1mol/L HCl) was the desorbing solution, velocity of flow was 1BV/h (BV-column bed volume).

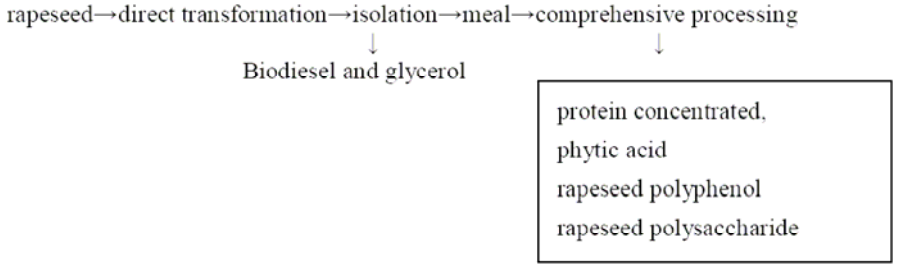
2.3.2 After primary purification as 1.3.1, we used chromatography column with Sephadex LH-20 to for further purification. Subfraction-1of polyphenol could be got with the decolourizer of methanol: H_2O=1:3 while subfraction-2 could be got with the decolourzer of methanol: H_2O=1:1.

3. Studies on the process route of comprehensive utilization of rapeseed

China has the second greatest consumption of petroleum. It reached 245.7 million tons in 2002, exceeding Japan and just behind America. In 2003, China consumed 83 million tons diesel oil and 40.16 million tons gasoline, 32% of the total consumption of petroleum was imported. In 2004, domestic consumption of energy sources increased at an average annual rate of 15% while the amount of domestic petroleum output only 1.67%. Therefore, the lack of energy sources will hinder the development of mankind.

Biodiesel, namely longchain fatty acid methyl ester from vegetable oils or animal fats, is a kind of renewable and environmental protection energy source, which can substitute for diesel. Biodiesel can not be produced cosmically in China mainly because its production cost is higher than the market price. So, we must explore the new thoughts and methods on the production of biodiesel to reduce its production cost.

Rape is the most preponderant oil plants crop in China, which is planted widely. Its yield reached 12 million ton in 2005, ranked first in the world. Therefore, we have found a unique technological route to produce biodiesel directly from rapeseed with the further comprehensive processing of rapeseed meal. The route was as follows:



The characteristics of route are following:

A. Abandon the conventional technological route with two steps at present which the oils are squeezed firstly from rapeseed and then transformed to biodiesel. The new route is to directly extract and transform the oils simultaneously, which change two steps into one step. So, the expenditure of pressing oil was decreased and then the cost of biodiesel product can be reduced greatly.

B. The highly active solid catalyst for ester transformation is used during the transformation of biodiesel. So, the disposal cost of KOH or NaOH alkalescent catalysis used in conventional production of biodiesel is reduced and the environmental pollution can be avoided too.

C. The continuum production of biodiesel and online exclusion of glycerol are implemented by means of the technology of constant methyl esterification at normal pressure, the products of biodiesel and glycerol with high quality will be obtained.

D. The quality of protein in rapeseed meal after rapeseed transformed directly to biodiesel was much better than that in the rapeseed meal squeezed conventionally from rapeseed because the former was obtained at the temperature being lower than 70°C. For example, the content of lysine in the former is doubled that of the latter. The feeding Bioavailability(biological

value) is further high and more benefit for the development of rapeseed meal.

E. The rapeseed meal byproduct after rapeseed transformed to biodiesel was used to extract and isolate the impurity in rapeseed meal such as glucosinolate, phytic acid, polyphenol, polysaccharide, cellulose etc by water solvent in divided step, the feeding protein concentrated was obtained rather than by the classical preparation method of protein concentrated from extracted protein. Simultaneously rapeseed polysaccharide, rapeseed polyphenol and phytic acid were recovered and prepared from the waste water. The four products including feeding protein concentrated, phytic acid, rapeseed polyphenol and rapeseed polysaccharide were obtained in the technological route. The output value and profit of these products were several ten times higher than that of rapeseed meal.

The development of rape biodiesel and comprehensive processing of rapeseed meal can lighten the lack of energy sources, and resolve the low profit of oil corporation as well which produces only oil and rapeseed meal such singleness variety of product. The increase of enterprise benefit may raise the purchase price of rapeseed so that the income of peasant and the enthusiasm of peasant planting rape will increase. It is significant to adjust the industry situation of countryside in China, promote the development of countryside economic, and walk out a way making the peasant affluent which transforms from agricultural products to industry products.

At present, the above comprehensive processing of rapeseed meal have successfully applied in industrialization production. The testing of biodiesel transformed directly from rapeseed has finished. The product line of biodiesel with 500 tons annual production had been built already. We are looking forward to researching and developing rapeseed together with our colleagues.

References

1. Wu Moucheng, Yuan Junhua, Shao Jinhua, Zhang Yan. “Studies of Comprehensive Processing and Utilization of Rapeseed”. Journal of Huazhong Agricultural University. 1999. 18(6).589-591
2. Wu Moucheng, Zeng Xiaobo et al. Research Progress on Processing and Comprehensive Utilization of Rapeseed. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P287-291.
3. Wu Moucheng Shao Jinhua, et al. Preparing Rapeseed Protein Isolate from Dehulled Rapeseed Meal which Oil was Extracted at Proper Temperature. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P306-312..
4. Zeng Xiaobo, Wu Moucheng et al. Preparation of Rapeseed Protain Concentrate by Washing with Acetone Solvent. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P299-305..
5. Wu Moucheng, Zeng Xiaobo et al. Study on Removal of Glucosinolates, Tannins and Phytates from Rapeseed Meal. Proceedings of International Symposium on Rapeseed Sciences. Science Press New York.2001. P292-298..
6. Wu Moucheng,Zeng Xiaobo. Studies on preparation and its functional properties of rapeseed protein concentrate. 11th International Rapeseed Congress, Copenhagen, Denmark. Abstracts, 450
7. Xue Zhaohui, Yin Jingzhang, Wu Moucheng, Yuan Junhua. Preparation and antihypertensive effect of rapeseed peptide (RSP). 11th International Rapeseed Congress, Copenhagen, Denmark. proceedings vilime 2 of 4, 689-693
8. Wu Moucheng, Zeng Xiaobo, Yuan Juan. Inhibition effect of rapeseed peptides (RSP) on tumor. 11th International Rapeseed Congress, Copenhagen, Denmark. proceedings vilime 2 of 4, 586-591
9. Xue Zhaohui, Yin Jingzhang, Wu Moucheng, The hydrolysis of rapeseed albumin with alcalase. 11th International Rapeseed Congress, Copenhagen, Denmark. proceedings vilime 2 of 4, 592-598
10. Xue Zhaohui, Wu Moucheng, Yin Jingzhang, Ruan Zheng, Isolation Characterization of Defatted Hua-Za 3 Meal protein. Food Science, 2004, 25(4):25-28
11. Zhou Quancheng, Wu Moucheng. Concentration of tocopherols by supercritical carbon dioxide with cosolvents. European Food Research and Technology. 2004, 219:398-402(SCI source)

A non-organic solvent process for the efficient recovery of canola oil

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Abstract

Conventional processing of canola (*Brassica napus*) generally uses either, a combination of seed expelling plus organic solvent based oil extraction, or non-organic solvent processes that are based solely on expelling of the seed. Organic solvent based oil extraction is highly efficient (>96% oil recovery) in comparison to non-organic solvent based expeller only processes (generally 80% oil recovery). As such solvent extraction processes tend to dominate. However, environmental and safety concerns with the use of organic solvents are such that non-organic solvent based processes would be preferred if the efficiency of oil recovery approached that of organic solvent extraction. MCN BioProducts Inc. has developed a proprietary non-organic solvent process for high efficiency recovery of canola oil in the form of free oil and oil contained in a high valued concentrate of insoluble protein. The process uses expellers for a front end mechanical de-oiling as typical for conventional non-solvent processes. The cake containing the residual oil is then slurried in water and processed through an aqueous fractionation scheme that generates a fibrous fraction, a concentrate of insoluble protein and a liquid stream. Conditions are set such that 20% of the residual oil in the expelled cake is directed to the low valued fibrous production, 20% to the high valued insoluble protein concentrate and 60% to the liquid stream. The liquid stream is then processed through an oil-water separation with the generation of free oil that is mixed with expelled oil. The net result is 92% recovery of whole seed oil as free oil plus 4% recovery of oil within the high valued protein concentrate product. As such the combination of conventional oilseed expelling plus aqueous oil extraction results in a net oil recovery as high valued products that approaches that of organic-solvent based extraction processes.

Introduction

Historically, canola (Brassica napus) has been grown primarily for the oil. The oil comprises approximately 40% of the seed weight, however, the high value of the oil relative to the low value of the meal are such that the oil provides 70% or higher of the revenues generated from a commercial seed crushing and solvent extraction process. As such crushing operations have focused on maximizing the efficiency of oil recovery. A typical pre-press plus solvent extraction process can provide >96% oil recovery. This compares favorably to the 80-90% oil recovery that is typically obtained with a non-solvent expeller only based process. This substantial difference in oil recovery has lead to the predominance of solvent extraction plants for the processing of canola seed. However, safety, environmental and product quality concerns associated with the use of organic solvents are such that non-solvent based processes would be preferred provided that oil recovery are not overly compromised.

One approach to improve the efficiency of oil recovery from a non-solvent expeller process is to process the oil-expelled cake through an additional aqueous fractionation process designed to generated high valued protein concentrates. Such a process has the potential to facilitate recovery of additional oil in the form of valued free oil or valued oil-containing protein concentrate. MCN BioProducts Inc has developed a proprietary process for the aqueous fractionation of non-solvent expelled canola cake to generate a high valued protein concentrates. To improve the overall efficiency of oil recovery from whole seed starting material, an oil-water seperation step was integrated into the basic aqueous fractionation scheme. By quantifying the crude fat contents of the various streams generated by the process it was possible to measure the flow of oil to these streams and thus quantify the efficiency of oil recovery as high-valued outputs.

Materials and Methods

Whole seed canola was obtained from a local supplier near Saskatoon, Saskatchewan Canada. The seed was transported to the pilot oilseed processing facilities of Agricultural Research Center of the USDA in Peroria Illonios USA. The seed was conditioned for 15-20 minutes at 85-90°C (French Oil Mill Machinery Model 324 – 3 deck 24 inch diameter) and the expelled through a pilot scale full press (French Oil Mill Machinery Model L-250 3.5 inch barrel). The press cake was returned to the process development centre of MCN BioProducts in Saskatoon.

The press cake was initially slurried in water and then processed through the proprietary MCN aqueous fraction process. The core process outputs 3 intermediate streams consisting of a low value hull-enriched by-product stream, a high valued insoluble protein concentrate, and a solids-free liquid stream in the form of an oil-in-water emulsion.

The liquid stream was then processed through a 3-phase disk-stacked oil separating centrifuge (Westfalia) to separate the free oil from the oil-in-water emulsion.

Dry matter and crude fat mass flows were monitored through the process. Dry matter was determined by weight differential upon evaporation of moisture using an HB43 Halogen Automated Moisture Analyzer. Crude fat was determined by solvent extraction using an Ankon XT20 automated fat analyzer.

Results

Oil Expelling. A total of 22 replicate runs of seed conditioning and expelling were conducted using the pilot scale

equipment at USDA in Peoria. The mean residual oil content of the full press cake obtained from 22 replicate runs was 9.39±1.09% of cake dry matter. This calculates to an 86.9±1.71% oil expelling efficiency from the starting seed.

Aqueous Fractionation. Water is added to the press cake to generate a slurry that is then processed through the core proprietary fractionation scheme. The process generates a first fraction enriched in hull fragments, a second fraction enriched in insoluble protein and a third fraction consisting of a solids-free liquid stream. Table 1 summarizes dry matter, protein and crude fat flux through the core fractionation process.

Table 1. Dry matter, protein and crude fat flows through the aqueous fractionation process*.

	Composition (% of d.m.)		Mass flows (% of starting cake)		
	Protein	**Crude fat**	**d.m.**	**Protein**	**Crude fat**
Starting cake	39.8	12.4			
Hull fraction	31.8±1.0	6.25±0.9	26.3±1.7	21.3±1.4	14.5±1.0
Protein concentrate	61.9±1.1	10.0±0.8	36.2±4.6	55.5±7.3	33.2±6.6
Liquid stream	13.1±0.4	12.0±1.0	36.9±3.1	11.9±1.0	40.4±6.4
Total % recovery			99.4	88.7	88.1

*Results are expressed as the mean and standard deviation obtained from triplicate runs of the proprietary fractionation process.

The hull fraction contained 6.3% crude fat on a dry matter basis and accounted for an average of 14.5% of crude fat in the starting expelled cake. The protein concentrate contained 62% protein and 10% crude fat on a dry matter basis and accounted for 33% of the starting expelled cake crude fat. The clarified liquid stream is the remaining fraction and this stream contained an average of 40% of the crude fat originating from the starting cake.

Crude Fat Recovery form the Liquid Stream. The clarified liquid stream contained an average of 41% of the crude fat in the starting press cake which is equivalent to 5.4% of the crude fat in the whole seed starting material. The liquid stream had the appearance of a solids-free cloudy milk-like material. Inspection of the liquid stream under light microscopy revealed an oil-in-water emulsion (Fig 1). Oil droplet size varied between 1-15 microns. Based on the distribution of droplet sizes an estimated 90% of the oil is contained in droplets of greater than 8 microns in diameter.

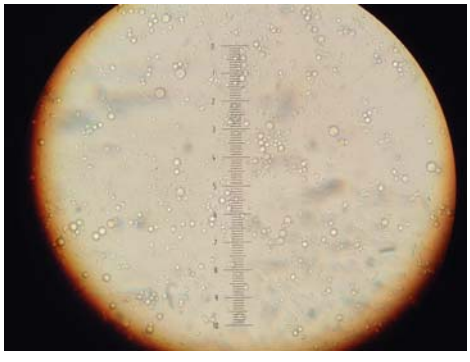

Fig 1. Light microscopy image showing the liquid stream fraction as an oil-in-water emulsion. Units are 23 µm and subunits are 2.3 µm.

Table 3 summarizes the result obtained in processing the oil-in-water liquid stream through a 3-phase disk-stacked centrifuge. The light out phase from the centrifuge averaged 40.8% crude fat, 5.3% non-oil dry matter in solution and 53.9% water. This stream accounted for 84.8% of the crude fat in the original feed to the centrifuge. The heavy out liquid phase was a largely aqueous stream with an average of 4.07% non-oil soluble dry matter and 0.13% crude fat. This stream accounted for an estimated 13.6% of the crude fat in the feed to the centrifuge. The sludge component of the 3-phase separation accounted for the remainder of the crude fat, water and non-fat dry matter.

Table 3. 3-Phase Disk-Stacked Centrifuge* Separation of Liquid Stream from Aqueous Fractionation of Expelled Canola

Fraction	% Dry Matter	% of total Volume	% crude fat		% crude fat recovery
			% as-is	% of d.m.	
Liquid stream input	4.62	100	0.77	16.78	
Light phase	46.1±9.8	1.6	40.8±3.2	88.5±0.53	84.8
Heavy phase	4.20±0.06	75.3	0.13±0.23	3.06±0.38	13.6
Sludge phase	7.17	7.4	0.45	6.25	4.3
Total % of input	95.9	84.3			102.7

*Continuous flow 3-phase separation on disk-stacked centrifuge. Dry matter and crude fat contents of light phase and heavy phase liquid outputs shown as the mean±S.D. of samples taken at the early, middle and late stages of the run. Volumes of light phase, heavy phase and sludge phase determined at the end of run. Sludge sampled at end of the run.

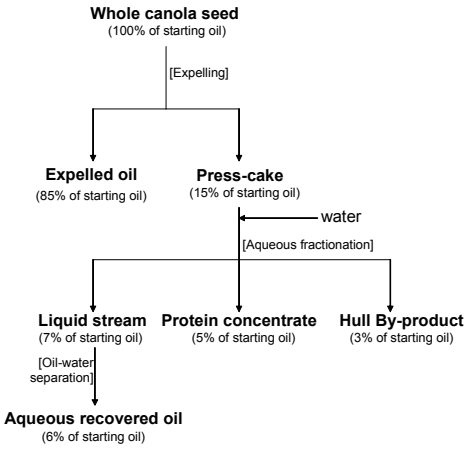

Fig 2. Projected oil flows through a non-solvent expeller plus aqueous fractionation and oil recovery process. Estimated recovery of oil as valued free oil plus protein concentrate = 96%.

Discussion

Current alternatives to solvent extraction for the processing of whole seed canola are disadvantaged by an 80-90% oil recovery relative to the near complete oil recovery obtained in solvent based oil extraction. To be competitive, a non-solvent process must provide comparable recovery of oil in the form of high valued products.

This paper described the flow of oil from expeller cake to the 3 fractionations that are produced by a novel proprietary aqueous fractionation process. In the process 15% of the crude fat in the expelled cake was directed to the low valued hull stream. The oil associated with the insoluble protein concentrate will have a comparable value to free oil given the high value of the protein in this stream while the majority of the oil in the liquid stream can be readily recovered by established oil-water separation processes. Figure 2 provides a projected oil mass balance for the integrated process. The net result is an overall 96% efficiency of oil recovery in the form of valued free oil plus oil within a high valued protein concentrate. As such oil recovery in this process is comparable to a conventional solvent based process.

New process of dehulling- cold pressing-expansion for double-low rapeseed

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Abstract

Rapeseed dehuller, twin-screw press and expander were assembled to a new process for double-low rapeseed. The technical and economic feasibility of the new process were analyzed. It can reduce conversion cost, improve quality of oil and meal and increase economic benefit. The conversion cost of new process is 142.85 RMB Yuan·t^{-1}, which is less than pre-pressing extraction process by 20%. Cold pressing oil meets the standard of the second grade rapeseed oil (Chinese national standard, GB 1536-1986). Rapeseed meal meets the Standard of the first grade Low-glucosinolate Rapeseed Meal for Fodder of standard enacted by Chinese Ministry of agriculture (NY 417-2000), which protein content is higher than 46%. The new process will achieve higher profit, which is promising in industrialization and application.

Key words: Rape, Double-low rapeseed,Dehulling, Cold pressing, Expansion, Process

Introduction

Oilseed Rape has become the third oil crops in yield all over the world (Wang, 2004). That content of glucosinate and erucic acid was decreased greatly in double-low rapeseed improved its nutritional value of oil and protein (Bell, 1982), which caused rapeseed changed from simple provider of oil to multi-purpose sources for oils, feedstuff and protein. At present, the double-low rapeseed was still processed by the traditional method, pre-pressing and extraction, which needed the hull, long time and high temperature. So obtained rapeseed meal had a lower content of protein, deep color and bad taste for fodder. Moreover, available amino acids of protein were destroyed evidently, while effected the using value of rapeseed meal seriously, and obtained crude oils had a bad quality which increased product cost and refining loss (Watrins et al., 1989; Egon, 1975; Elizabeth et al., 1991). Traditional process of pre-pressing and extraction didn't realize excellent utilization for double-low rapeseed and made source further wasted, which already had been not fit for processing of double-low rapeseed(Zheng, 2001).

For realizing high efficient processing of double-low rapeseed, a great deal of new technology of rapeseed processing were studied, such as dehulling, cold pressing and expansion were the popular study subjects(Huang, 2002). Dehulling rapeseed not only increased content of protein, effective value of feed, and improved the meal taste for feed, but also meliorate oil's quality and decreased refining cost(Ragnar, 1992; Huang et al., 2000); Cold pressing could obtain green natural good quality rapeseed oil, avoid loss of available amino acids, increased nutritional effective value of meal and observably decreased the energy expending of rapeseed processing(Huang, 2002; Rasehorn et al., 2000); Expansion could improve materiel's extraction character, speed up extraction of oil, advance yield of oil and decrease production cost(Watrins et al., 1989; Wang, 2001). At present, the key equipments had been developed, such as dehulling, cold pressing and expansion machine (Huang et al., 2000;Rasehorn et al., 2000;Watrins et al., 1989; Lusas & Watkins, 1990). On the side of new processing for rapeseed, a factory, which could produce high quality cold-pressing oils, was been built in Germany (Rasehorn et al., 2000), but obtained cold-pressing meal had a bad extraction character. In 2003, the new process of dehulling-expansion for double-low rapeseed had been practiced(Li et al., 2004), and high protein content rapeseed meal been obtained, but high oil content of expansion meal increased the burthen of extraction system and made meal a high residual oil rate. Therefore, it is still difficult to popularize for the new process, dehulling cold- pressing and dehulling-expansion.

According to Double-low Rapeseed high effect producing actuality and developmental trend, a new idea, dehulling- cold pressing-expansion were assembled to a new process for double-low rapeseed, was given. The feasibility and economy of new process were analyzed.

1 Material and Method

1.1 Experimental material

Rapeseed was Double-low Rapeseed obtained from Wuhan Zhongpai oil Inc. The water content of rapeseed was 11.32%, impurity content 2.95%, oil content 38.68%.

1.2 Processing flow

The processing flow was showed as Fig 1.

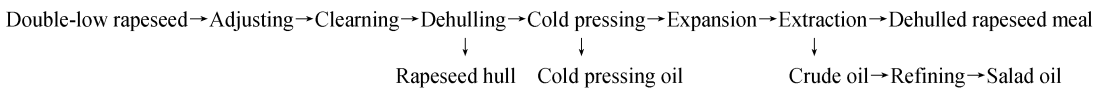

Fig. 1 The process flow diagram of dehulling-cold pressing–expansion for double-low rapeseed

1.3 Key equipments

YTTP-75 rapeseed dehuller; SSYZ50 twin-screw press; PHJZ-100 expander.

1.4 Operation conditions

1.4.1 Adjusting

Because the water content was high in new rapeseed while there was some requirement to water content in dehulling process, drying tower etc. equipment was adopted to adjust water content to 8-10%.

1.4.2 Cleanning

Dehulling-cold pressing-expansion process had high requirement in iron-excluding process. Magnetic roller was adopted to exclude iron impurity. During adjusting, double-low rapeseed was processed in vibration cleaning screen, dusty-excluding fan, and magnetic roller, in the end the rate of impurity content reached the requirement of lower than 0.5%.

1.4.3 Dehulling and separation

The appropriate moisture content for rapeseed de-hulling was 8-10%. In order to get good effect of de-hulling, rotation speed was adjusted to about 1500r·min^{-1} by transducer, and then the transducer was adjusted tinily according to observation of de-hulling rate and powder degree. Hull and kernel were separated by adjusting the wind quantity of fan, vibration speed of screen and the height of equipment of sucking hull or kernel. Hull was collected and deposited separately, and kernel was sent to next cold pressing process.

1.4.4 Cold pressing

At the beginning of press, increased feedstock gradually by adjusting frequency converter, so the press in barrel would increase gradually. Twin-screw press would work normally when current of main electromotor reached 40-50A. In pressing process, feedstock was delivered with equilibrium. If feedstock flow decreased, the pressure in barrel would fall, consequently oil yield would fall too. In the end of cold pressing process, cold pressing oil and cold pressed cake were obtained. Cold pressing oil was purified through plate filter and then was canned to get the product cold pressing rapeseed oil. And cold pressed cake was sent to next expansion process.

1.4.5 Expansion

Cold pressed cake was heated to the temperature of 90°C in cooker and then sent to expander. Feedstock flew was controlled by frequency converter to keep current between 100-120A. During expansion process, the temperature of expansion canister was adjusted directly by 0.5-0.8Mpa steam to keep it about 110°C. Attention should be paid to ensure steady feedstock flow in the process of expansion. If feedstock flow decreased or even stopped, press in expansion machine would fall and impact the expansion effect.

1.4.6 Extraction

Expansion materials were delivered to rotating extractor by a screw conveyor. The solvent infiltration speed and solvent dripping capability of expansion feedstock were better than that of pre-pressed cake, so extracting velocity was improved. Rotation speed could be increased comparatively. Oil mixture was desolventized in a series of evaporators and stripping columns. Crud oil gained by extraction was sent to refining plant and salad oil was made out through routine process of degumming, bleaching and deodorization. Wet rapeseed meal with solvent was desolventized by DT desolventizer-toaster, and then was cooled and packed to get dehulled double-low rapeseed meal.

1.5 Determining methods for physical analysis

Lipidic acidity, color and luster, peroxide value, heating test, odor and taste, moisture, volatile materials, impurity, the oil content, water, impurity of rapeseed, and crude protein, ash, crude fiber, crude fat, etc. of rapeseed meal were measured with Chinese national standard method. ITC and OZT content in rapeseed meal were analyzed by using Chinese national standard method, such as GB/T13087-1991 and GB/T13089-1991 separately. Component of amino acid in rapeseed meal was analyzed with the method of NY/T793-2004. Other process parameters were analyzed according 《Standard complication of cereals and oils. Determination methods》 SB/T10134－92 determination regulations of vegetable oil producing technique (No 1 Compiling Room of Standard Press of China,1998).

2 Results and analysis

2.1 The technical parameters of dehulling-cold pressing-expansion process for double-low rapeseed

The technical parameters of each procedure in dehulling-cold pressing-expansion process for double-low rapeseed were measured, and results were as follows:

When rapeseed was dehulled, oil contained in kernel increased to about 45%, and crude fiber content decreased a lot to 3-5%. So the phenomena of no oil out and shapeless cake would appear when present ZX18 or ZY24 single screw press was adopted. The main reason was that the dehulled rapeseed contained high oil and low crude fiber. Friction was so small that press was hard to be formed. Two screws structure was adopted in twin-screw press with a principle of combination of

joggling and non-joggling. In the press barrel, multiple-stage compression and relaxation and a thin layer of materials when pressing were applied. Compared with traditional single-screw press, twin-screw press provided high compress ratio which could reach 23 in theory, increased ratio of length and diameter of pressing obviously, and prolonged pressing time, so it could press completely. Residual oil of cold pressed cake of dehulled rapeseed which was processed in twin-screw press was about 15% which corresponded to that of hot pressed cake gained in pre-pressing process. The problem of cold pressing technology of dehulled rapeseed was solved. Compared with Germany single-screw equipment, the rate of residual oil of cold pressed cake corresponded, but the electric machine capacity of twin-screw press was only 1/3 as to the same capacity Germany equipment (Rasehorn et al., 2000).

Table 1 The technical parameters

Index	Value
Dehulling rate(%)	95.86
Residual oil of cold pressed cake (%)	15.08
Expansion rate	1:1.69
Density of expansed materials ($kg \cdot m^{-3}$)	488.56
Residual oil of rapeseed meal (%)	0.75
Capacity($t \cdot d^{-1}$)	62

PHJZ-100 expander was taken in expansion procedure for cold pressed cake. Expansed materials were porous particles with expansion rate 1:1.69 and density of 488.56$kg \cdot m^{-3}$. Feedstock was grinded, kneaded and crushed furiously in expansion machine, so cell tissue structure was destroyed completely and extraction property was improved obviously. After extraction, residual oil in rapeseed meal was 0.75% which fulfilled the requirement that the rate of residual oil in rapeseed oil should less than 1%.

2.2 Product quality of dehulling-cold pressing-expansion for double-low rapeseed

2.2.1 Quality of cold pressing oil from double-low rapeseed

Solubility of non-triglycerides component in lipid was low(Elizabeth et al., 1991), because rapeseed hull which was rich in pigment had been excluded and the temperature of cold pressing was low. Cold pressing oil gained from practical producing line was tested by Center of Quality Inspection & Test for Oil Crops Products Agricultural Ministry of China and the results were show in table 2. It was clear that the indexes of color and acidity etc. of this process were much better than that of pre-pressing crude oil. This product meeted the standard of the second grade rapeseed oil (Chinese national standard, GB 1536-1986), and could be edible when it was simply treated by filtration to exclude solid impurity. The refining processes of degumming, caustic refining, bleaching, deodorization etc. could be reduced. So, the contamination of organic chemical reagent and the appearance of trans-fatty acid could be avoided, and VE and other natural component were kept. This product could be treated as green natural healthy food, which greatly improved the economical value.

Table 2 The quality of cold pressed double-low rapeseed oil

Index	The second grade rapeseed oil	Cold pressing rapeseed oil
Color and luster (Trough thickness 25.4mm)	≤Y35 R7.0	Y35 R4.0
Odor and taste	Having the inherent flavor and taste of rapeseed oil, no peculiar smell	Having the inherent flavor and taste of rapeseed oil, no peculiar smell
Acidity(mg $KOH \cdot g^{-1}$)	≤4.0	1.1
Moisture content and volatile materials(%)	≤0.10	0.04
Impurity(%)	≤0.10	0.01
Heating test(280°C)	The color allow to be deepen and not to be darken. Trace matter allow to be separate out.	The color become deepen. No matter is separate out.

Table 3 The comparison of the quality of different rapeseed meal

Index	National I Low-glucosinolate Rapeseed Meal for Fodder	Dehulled double-low Rapeseed Meal	Rapeseed Meal
ITC+OZT($mg \cdot kg^{-1}$)	≤4000	3840	-
Crude protein(N × 6.25,%)	≥40.0	46.8	38.6
Crude fiber(%)	＜14.0	5.6	11.8
Crude ash (%)	＜8.0	7.8	7.3
Crude fat(%)	-	0.95	1.40
Moisture content(%)	＜12.0	11.2	11.5
Lysine(%)	-	2.14	1.30

Date come from Eleventh Edition Chinese Feed Database

2.2.2 Quality of dehulled double-low rapeseed meal

The quality of rapeseed meal got from dehulling-cold pressing-expansion process for double-low rapeseed was improved obviously, which meet the Standard of the first grade Low-glucosinolate Rapeseed Meal for Fodder of standard enacted by Chinese Ministry of agriculture (NY 417-2000). Due to protein content in dehulled double-low rapeseed meal increased obviously, anti-nutrition factors decreased greatly, taste was improved and had high value as fodder, all of which made the

accession amount of dehulled double-low rapeseed meal into fodder increase. This rapeseed meal could also be processed to get concentrated protein, which would increase the economical value of rapeseed meal.

2.3 Production cost of dehulling-cold pressing-expansion process for double-low rapeseed

Production cost of dehulling-cold pressing-expansion process for double-low rapeseed was shown in table 4. Power consumption of per feedstock increased for adding the equipment of dehuller and expander. However, in this new process steam consumption decreased greatly for the procedure of cooking was saved. Because the solvent infiltration property and solvent dripping capability were good, solvent content in wet rapeseed meal decreased, density of mixed oil increased. Compared with pre-pressing process, steam consumption and solvent consumption decreased greatly in the process of wet rapeseed desolventizing and mixed oil vaporizing and stripping.

Calculated the consumption of water, power, steam, solvent, refining cost, equipment depreciation and worker's salary etc., the production cost in total of dehulling-cold pressing-expansion process was 142.85 RMB Yuan·t^{-1} which decreased by about 20% compared with pre-pressing extraction process.

Table 4 The comparison of production cost(50t·d^{-1})

Index	Dehulling-cold pressing–expansion process	Pre-pressing extraction process
Power consumption(kwh·t^{-1})	70	61
Water consumption(t·t^{-1})	0.50	0.70
Coal consumption (t·t^{-1})	0.05	0.07
Solvent consumption (kg·t^{-1})	5.0	6.0
Refining Cost (RMB Yuan·t^{-1})	7	37
Equipment investment (ten thousand RMB Yuan)	500	420
Equipment depreciation charge (RMB Yuan·t^{-1})	25	21
Worker's salary (RMB Yuan·t^{-1})	12	12
Management expenses (RMB Yuan·t^{-1})	20	20
Production cost (RMB Yuan·t^{-1})	142.85	175.87

3 Discussion

3.1 Although high quality cold pressing oil was gained by process of dehulling-cold pressing for double-low rapeseed, there was still about 15% fat in cake when it was used as fodder directly. Because rapeseed kernel was pressed directly without flaking and cooking, cell tissue of cold pressed feedstock was destroyed a little(Yiu et al.,1983), fat extraction velocity was low, solvent infiltration property and solvent dripping capability of solvent were bad, and residual oil rate of rapeseed meal was high when extracted directly. Solvent consumption and steam consumption rose, which made product cost increase. Problem of producing oil by extraction from cold pressed cake remained to be solved. Though high-protein rapeseed meal was gained in dehulling-expansion process for double-low rapeseed, oil content in expansion feedstock was as high as 28% (Li et al., 2004) which affected the producing ability of extraction machine and made the resident oil rate of extraction rapeseed meal increase. what was more, producing cost increased when solvent consumption rose and burthen of evaporation and evaporation system was strengthened. In addition, the extrusion crude oil gained by dehulling-expansion process must be refined to become edible for its bad quality, which increased processing cost and decreased the economical value of double-low rapeseed oil. Dehulling, cold pressing and expansion were assembled in this experiment to build new dehulling-cold pressing-expansion process. It could not only gain high quality cold pressing oil, but also expansion process solved the problem that it was difficult to extract to gain oil from cold pressed cake. Besides high quality dehulled rapeseed meal was gained. The process of dehulling-cold pressing-expansion was proved by practice to be feasible, and was awarded as Chinese invention patient (patent number: ZL 01 1 06583.4).

3.2 In dehulling-cold pressing-expansion process, about 0.15t rapeseed hull that was new source to be used was gained from per tone double-low rapeseed. By testing, protein content in rapeseed hull was 13%~16%, which was a bit higher than that in alfalfa that was planted in home and abroad as fodder. Related experiment were being carried out to made rapeseed hull take place of alfalfa as fodder for ruminant such as cattle and sheep etc.. When planted edible fungus with rapeseed hull instead of cotton hull, product increased greatly(Huang et al.,2000). Besides Wu Mou-cheng et al. carried out study on integrated utilization of rapeseed hull(Wu et al., 1999), and they separated and extracted fine chemical products such as vegetable polyphenol and phytic acid etc.. Function and application of these products were studied farther.

References

Bell J M. Nutrients and toxicants in rapeseed meal: a review. *Journal of Animal Science,*1982,58: 996-1010.

Egon J. Effects of variation of heat treatment conditions on the nutritional value of low-glucosinolate rapeseed meal. *Journal of the Science of Food and Agriculture,* 1975,26:157-164.

Elizabeth M P, Vivekenand S V, Frank W S. Effect of heat treatments on Canola Press Oils. *Journal of the American Oil Chemists' Society,* 1991, 68(6):401-406.

Huang F H. High profit processing and multiple value-added techniques of canola. *China Oils and Fats,* 2002,27(6):9-11.

Huang F H, Zhou L X, Li W L, Wang J W, Lu S G, Cheng X Y. Study on dry dehulling technology of rapeseed. *China Oils and Fats,* 2000,25(6):48-49.

Huang F H, Zhang X J, Zhang Y B, Liu M Y, Li W L. A study of culturing mushroom with rapeseed coat. *Chinese Journal of Oil Crops Sciences,* 2000,22(4):37-39.

Li W L, Huang F H, Wang X L. Research and Application in Double-low Rapeseed Dehulling, Expansion and Extraction. *China Oils and Fats,*

2004,29(8):9-12.
Lusas E W, Watkins L R. Edible Fats and Oils Processing, Basic Principles and Modern Practices. Champaign: AOCS Press, 1990:61.
No 1 Compiling Room of Standard Press of China. Department of Standard, Bureau of Scientific and Technical Quality, Ministry of Inland Trade of the People's Republic of China. Standard complication of cereals and oils. Determination methods. Standard Press of China, 1998,23-440.
Ragnar O. Modern processing of rapeseed. *Journal of the American Oil Chemists' Society,* 1992,69(3):195-198.
Rasehorn H J, Deicke H D, Xin Y M. Theory and praxis of decortication and cold pressing of rapeseed. *China Oils and Fats,* 2000,25(6):50-54.
Wang E H. Brief introduction of expansion pretreatment in oil industry. *Sciences and Technology of Cereals, Oils and Foods,* 2001,9(3):29-30.
Wang H Z. Medium-term and long-term developing stratagem of variety improvement of China rape. *Chinese Journal of Oil Crops Sciences,*2004,26(2):98-101.
Ward J A. Pre-pressing of oil from rapeseed and sunflower. *Journal of the American Oil Chemists' Society,* 1984,61(8): 1358-1361.
Watrins L R, Johnson W H, Doty S C..Expander process for oilseeds improves extraction and reduces energy requirements. *Oil Mill Gajetteer,* 1989,94(8):30-34.
Wu M C, Yuan J H, Shao J H, Zhang Y. Studies of comprehensive processing and utilization of rapeseed. *Journal of Huazhong Agricultural University,* 1999,18(6):589-591.
Yiu S H, Fulcher R G, Altosaar I. Processing effects on the structure and microchemical organization of rapeseed and its products. 6th International Rapeseed Conference, Paris, 1983,1490-1495.
Zheng J C. Discussion of oil extraction technology for high quality rapeseed. *China Oils and Fats,* 2001,26(5):38-39.

The influence of processing conditions on the nutritive value of canola meal

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Canola production in Australia

Australia produces around 1.5 million tonnes of canola annually. In 2005 the canola harvest at 1,438,750 tonnes from 960,000 hectares was slightly lower than 2004. The yield varied from a state average of 1.4 t/ha in Western Australia (WA) to 1.8 t/ha in New South Wales (NSW). The national average yield is around 1.5 t/ha. Around 400,000 tonnes is crushed producing around 240,000 tonnes of meal. The meal produced is classified as expeller or solvent extracted meal with approximately 85% of the meal processed being solvent extracted. Mechanically extracted expeller meal contains around 10% oil whereas solvent extracted meal has around 1%.

Australian Canola Quality

Australian canola is grown over a vast range of environments from northern NSW to the southern parts of WA. As a result there is a significant range in quality, particularly for oil and protein contents. Fatty acid profiles, chlorophyll levels, free fatty acids and other oil characteristics can also vary with growing conditions. The average quality of Australian canola is presented in Table 2.

Table 1: Average quality data of Australian canola seed 2005 (McFadden, et al. 2005)

Quality Parameter	Mean
Oil content, % in whole seed @ 6 % moisture	42.2
Protein content, % in oil-free meal @ 10 % moisture	36.3
Glucosinolates, µmoles/g in whole seed @ 6 % moisture	7
Volumetric grain weights (kg/hL)	64.1
Oleic acid concentration (C18:1), % in oil	60.9
Linoleic acid concentration (C18:2), % in oil	19.9
Linolenic acid concentration (C18:3), % in oil	10.8
Erucic acid concentration (C22:1), % in oil	0.1
Saturated fatty acid concentration, % in oil	7.0
Iodine Value	116.2

Canola meal is well recognised as a high protein source for use in stock feed. The high protein meal has a well balanced amino acid profile. Depending on processing conditions the meal may also contain a substantial amount of high energy canola oil which adds to the meal quality. The amount of oil and the fatty acid profile will be influenced by the method of extraction.

As well as the beneficial components of canola meal, it also has some limitations. Initially rapeseed, from which canola was developed, contained high levels of antinutritional components. Traditional meal, contained up to 150 µmols/g of glucosinolates as well as tannins, sinapine and phytic acid all of which influence meal quality.

Protein Content: The average canola meal protein content in 2005 was 36.3 %. This is relatively low when compared to previous years (Fig1.). Protein concentration has an inverse relationship to oil content with the maximum protein achieved in years when oil content is very low. In 2004, crops were badly stressed with drought conditions producing low oil but very high protein (Fig 1).

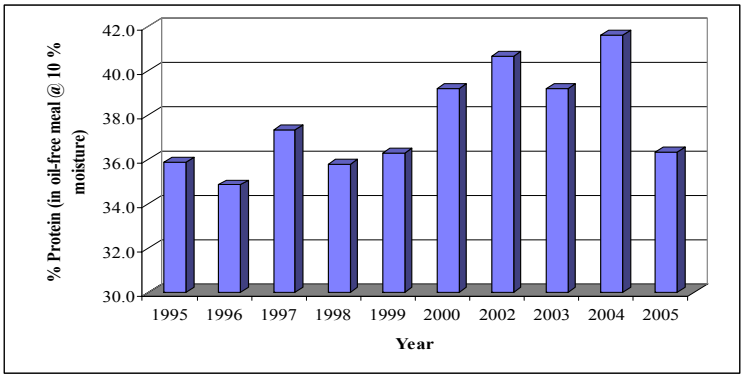


Figure 1: **Australian protein content in canola meal 1995-2005** (McFadden, et al. 2005).

Glucosinolate Concentration: Glucosinolate concentration in Australian canola meal is very low relative to traditional types. In 2005 (McFadden, et al. 2005) concentration ranged from 3 to 12 μmoles/g of total glucosinolates. Some individual sites produce more glucosinolates, particularly under drought stress (Mailer and Cornish 1987).

Processing

Processing canola meal involves several steps each of which contributes to changes in the meal product. The nutritional value of the meal is influenced in particular by temperature, time and moisture. The stages of processing are outlined:

Preconditioning and Flaking: involves preheating the seed and passing it through a roller mill to physically rupture the seed coat without damaging oil quality.

Seed cooking: Cooking thermally ruptures the remaining oil cells at around 90°C. This serves to inactivate myrosinase enzyme and additionally adjust the moisture content for better oil extraction. The time taken is usually around 20 minutes and temperatures are between 80-105°C. Temperatures up to 120°C may be utilised to remove glucosinolates which otherwise impart an odour to the oil.

Pressing: Screw presses or expellers are used to physically squeeze the oil from the canola seed flakes. Around 60-70% of the oil is extracted but care needs to be taken not to damage the meal or presscake.

Solvent extraction: Presscake is solvent extracted to remove the remaining 15-20% oil using hexane. The solvent or miscella (oil and solvent) is sprayed onto the presscake and allowed to percolate through, removing the oil. The remaining meal with less than 1% oil and saturated with solvent is called marc.

Desolventising/Toasting: This process removes solvent from the marc using steam. The last stage of drying is done with dry heat of around 105°C. After about 20 minutes in the D/T process the meal contains around 1% oil and 15% moisture. Moisture content is adjusted to around 10% before transporting to storage containers.

Changes to meal quality

Protein: Meal quantity and quality are affected by various factors from growing, maturity and harvest but the major influences occur during processing. Excessive heating in particular can result in damage to protein and amino acids and reduced digestibility of amino acids, particularly lysine. This protein is referred to as by-pass protein. By reducing the protein digestibility this can benefit ruminants by allowing the protein, which may be lost in the rumen, to stay intact until it reaches the true stomach. However, it is necessary to control conditions to produce by-pass protein without protein degradation.

Crude fat: The oil content of the meal is important as it provides energy to the meal. The oil content varies depending on the method of extraction. Solvent extraction produces meal with around 1% oil whereas expeller may contain up to 12% oil. Cold press meal will contain even higher levels. Despite the increased value to the meal, it is uneconomical for the processor to leave oil in the meal as there is no premium paid for the oil content.

Because cold pressed meal has more oil than solvent extracted oil this results in other changes in meal components. It therefore has lower protein than expeller or solvent extracted meal and higher bulk density than solvent extracted meal due to the extra oil.

Glucosinolates: Processing temperatures can be adjusted in the early stages of processing to inactivate endogenous myrosinase enzyme which otherwise breaks down glucosinolates. If the seed is ruptured in the presence of moisture, glucosinolates will be rapidly decomposed releasing isothiocyanates, thiocyanates nitriles and oxazolidinethione. These by-products influence both meal and oil quality. Enzyme deactivation is achieved by heating the seed at 10% moisture to 80-90°C. The majority of glucosinolates remaining after oil extraction are removed by high temperature in the D/T process although the temperature needs to be controlled to avoid other damage to the meal. Glucosinolates are higher in cold pressed meal than in solvent extracted meal although glucosinolates are generally low compared to traditional canola and rapeseed.

Lysine loss: Amino acid digestibility is reduced through high temperature treatment of canola meal. There is considerable evidence to show that the method of meal processing has a major effect on lysine availability to stock. This is illustrated in Table 3 (van Barneveld, et al 1999). Availability of lysine in particular is reduced in the D/T process. Temperatures of 105°C which are commonly used (Newkirk and Classen 2000) significantly reduce crude protein and lysine

digestibility and reduce metabolizable energy.

Table 3. Comparison of extraction methods on canola meal composition

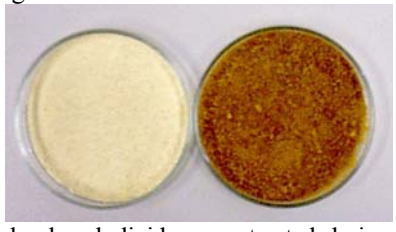
	Ingredient (g/kg)				
Nutrient	solvent extraction	expeller extraction	cold pressed	Seed	Seed (QAC)
Dry matter	882.1	951.3	913.3	949.1	940
Gross energy	177.5	206.0	227.1	272.0	
Crude protein	333.1	325.1	265.4	200.5	208.8
Crude fat	44.8	130.2	255.5	397.2	422
Crude fibre	126.8	148.6	156.5	165.9	
NDF	258.4	329.8	312.4	478.4	
ADF	162.7	234.9	186.0	383.5	
Ash	62.6	57.0	46.9	34.3	
Glucosinolates	1.4	3.0	11.1	9.8	7

Adapted from van Barneveld (1998) and McFadden et. al. (2005).

Table 3. The effect of processing on lysine availability in canola meal (adapted from van Barneveld et. al. 1999).

	Cold-pressed	Expeller	Solvent
Total Lysine	17.41	17.25	18.70
Reactive lysine	13.00	10.88	11.38
Reverted lysine 'loss'	25.30	36.90	39.10

Colour: Some feed manufacturers prefer a light coloured meal. High cooking temperatures at high moisture levels can cause significant darkening of canola meal. Temperatures above 110ºC did not cause significant changes to colour in dry meal in this study but considerable darkening occurred in meal with 10% moisture.



Gums and Soapstocks: Gums include phospholipids are extracted during crude oil degumming and soapstocks are recovered during alkali refining to remove free fatty acids. These compounds are often added back into the meal after the D/T process at around 1-2%, increasing the oil content and the metabolizable energy. It also helps to dampen the meal.

Tannins: Tannins include a range of products including sinapine. Sinapine has been found to cause undesirable flavours in eggs from poultry fed canola meal. Australian canola contains around 0.6 – 1.8% sinapine (unpublished results).

Minerals: are not influenced by methods of processing other than where gums or supplements are added back to the meal after processing.

Table 4. Amino acids showed little variation between processors (Rider Perez 2002)

Analysis	Newcastle solvent	Melbourne Solvent	Numurkah solvent	Pinjarra expeller
Dry matter %	90.5	89.2	89.6	90.2
Crude protein	414	419	418	335
Phosphorus	12	11.5	11.1	10.9
Calcium	8.4	7.0	6.8	7.5
Sulphur	7.0	7.2	7.0	7.0
Fat	49.4	30.7	55.4	129.3
Free condensed tannins	34.2	31.3	38	35.6
Bound condensed tannins	10.1	4.8	24	5.2
Total tannins	44.3	36.1	62	41.8
Sinapine	11.8	12.7	14.8	14.0
Glucosinolates (µm/g)	2	4	3	7
NDF	327.1	285.9	321.4	248.3
Layer hen AME (Mj/kg DM)	11.0	10.6	11.2	11.1
Layer hen AMEn	10.4	9.7	11.1	10.9
Broilers AME (Mj/kg DM)	8.7	9.2	9.7	11
Broilers AMEn	7.6	8.5	8.6	10.4

AME apparent metabolisable energy

Summary: Considerable work has been carried out in Canada and some in Australia on meal quality. However, canola meal is underutilised in Australia due to limited knowledge about the product quality. New data on processing effects

on meal quality are currently being accessed from processors throughout Australia and this will be presented at the 12th International Rapeseed Congress.

References

Hickling, D. 2001 Canola meal feed industry guide. 3rd edition. Canola Council of Canada.

Mailer, R.J. and P.S. Cornish, 1987. Effects of water stress on glucosinolates and oil content in the seeds of rape (*Brassica napus* L.)and turnip rape (*Brassica rapa* L. var. *silvestris* (Lam.) Briggs. *Australian Journal of Experimental Agriculture* 27:707-711.

McFadden, A., Mailer, R.J., and Parker, P. 2006. *Quality of Australian canola*. Australian Oilseed Federation publication ISSN 1322-9397.

Newkirk, R.W. and H.L. Classen. 2000. The effects of standard oil extraction and processing on the nutritional value of canola meal for broiler chickens. Poultry Science. 79(Suppl. 1):10.

Perez, Rider. 2002. Characterisation of and canola meal cottonseed meal at practical inclusion levels for use in broiler and layer diets. RIRDC Report DAQ-264 J.

van Barneveld, R. 1998. Influence of extraction method on the nutritive value of canola meal for growing pigs. South Australian Research and Development Institute.

van Barneveld, R.J., Ru, Y., Szarvas, S.R. and Wyatt, G.F. 1999. Effect of oil extraction process on the true ileal digestive reactive lysine content of canola meal. In Manipulating pig production VII p. 41. [P.D. Cranwell, editor] Australian Pig Science Association: Adelaide.

Improving rapeseed meal quality by reduction of condensed tannins

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Abstract

Condensed tannins can potentially have a major impact on animal nutrition, particularly because of their ability to form indigestible, astringent or bitter-tasting complexes with proteins. One option to overcome this problem is the breeding of yellow-seeded rapeseed with reduced condensed tannins in the seed coat. This might be achievable via selection of genotypes with smaller endothelium cells and consequently a spatial reduction in condensed tannin accumulation (seed coat structural cell mutants), or alternatively by selection of genotypes with reduced biosynthesis of condensed tannins (flavonoid biosynthesis mutants). Both types of *transparent testa* (*tt*) mutants are well-characterised in *Arabidopsis*, however the genetic basis of the yellow-seed trait in the polyploid genome of rapeseed is still not completely understood. As part of ongoing research into breeding of yellow-seeded rapeseed, the aim of this work is the development of analytical and screening procedures for condensed tannins in oilseed rape seeds, with a view towards isolation and characterisation of the responsible genes in yellow-seeded mutants.

Introduction

Plant tannins make up a distinctive group of high molecular weight phenolic compounds that have the ability to complex strongly with proteins, starch, cellulose and minerals. Chemically three groups of tannins are distinguishable: phlorotannins, hydrolysable and condensed tannins (*syn*. proanthocyanidins). The phlorotannins have been isolated from species in several genera of brown algae, whereas the hydrolysable and condensed tannins are widely distributed throughout the plant kingdom. Plant tissues containing tannins include bark, wood, fruit, seeds, leaves, roots and plant galls. Different groups of tannins have been associated with the maintenance of seed dormancy, while others have allelopathic and bactericidal properties. In rapeseed (*Brassica napus* L.) condensed tannins are largely responsible for the dark colour of the seed coat, where they accumulate predominantly in the endothelium cell layer between the outer integument and the aleurone layer. Whereas the proportion of condensed tannins in the cotyledons of *B. napus* seeds is comparatively low (only 0.1-0.5% of dry weight), condensed tannins in dark-seeded *B. napus* can comprise up to 6% of the seed coat. This means that they contribute significantly to rapeseed meal, with a total content of up to 800 mg/100g after oil extraction.

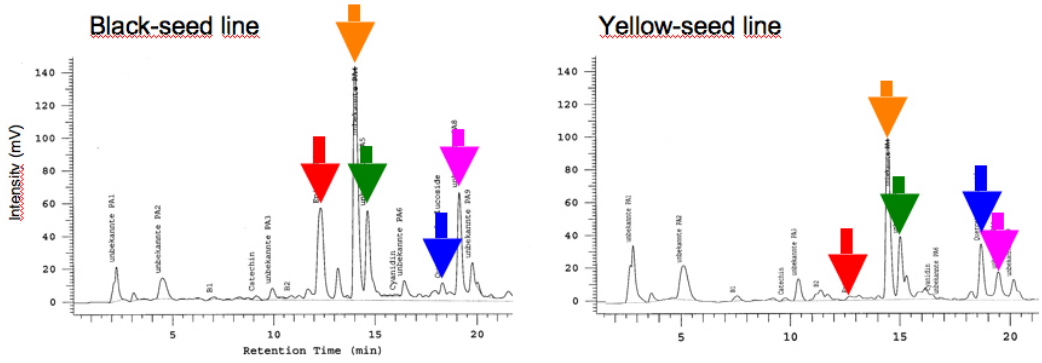
By localising quantitative trait loci (QTL) for condensed tannin content in *B. napus* seeds and comparing these to the positions of promising candidate *tt*-genes, we hope to develop closely-linked molecular markers for selection regarding important genes involved in the accumulation of antinutritive tannins in rapeseed meal.

Methods and Materials

A dense genetic map was generated from a population of 166 doubled-haploid lines derived from a cross between an inbred line of the black-seeded German winter oilseed rape cultivar 'Express' and the true-breeding, yellow-seeded line '1012/98', both with 00-seed quality. Significant QTL involved in seed colour, total falvonoids and in the contents of important flavonoid compounds were mapped usnig the software PLAB-QTL based on analyses of seeds grown in field trials in Einbeck and Gross Gerau, Germany from 2003-2005. Seed colour was measured qunatitatively based on digital reflectance values. Total condensed tannins were estimated by spectrometry based on the vanillin assay, while individual flavonoid compounds were quantified via HPLC using standards to identify peaks.

Results

Large differences were found between the black- and yellow-seeded mapping parents in total condensed tannin content and in different flavonoid compounds measured by HPLC (Figure 1). In particular the yellow-seeded line showed an absence of epicatechin, which in Arabidopsis is thought to be the major precursor of condensed tannins in the seed coat (Lepiniec et al. 2006). However the correlation of total flavonoid content with seed colour was comparatively low (Figure 2). This suggests that a significant proportion of the total seed flavonoids are non-coloured flavonoids that do not influence the seed colour but may still have a nutritional effect by forming indigestible compounds with proteins, for example.



PA peak	Black seed	Yellow seed
Epicatechin	high	reduced (down to zero)
Unknown PA4	very high	reduced
Unknown PA5	high	reduced
Quercitin-3-glucoside	very low	increased
Unknown PA8	high	reduced

Figure 1: HPLC measurements of flavonoid compounds in black and yellow seeded lines allowed the identification of components with large differences in levels associated with yellow seed colour. In particular epicatechin, a precursor of condensed tannins, was absent in the parental yellow-seeded line, while quercitin-3-glucoside was significantly increased compared to the black-seeded parent. A number of unidentifiable proanthocyanidins, presumably including condensed tannins, also showed significant reductions in yellow-seed lines.

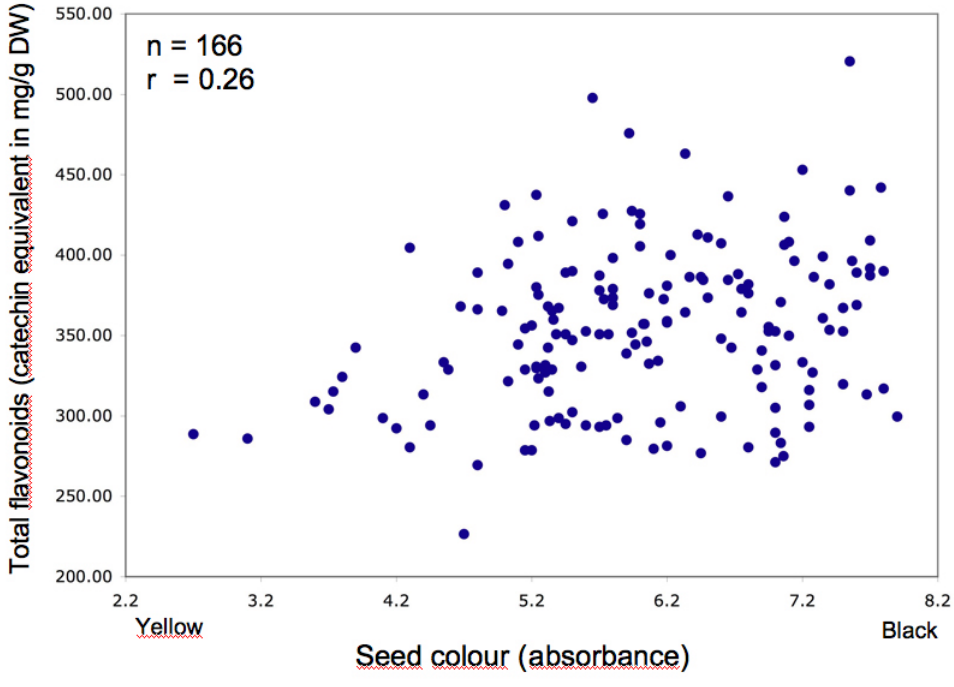


Figure 2: Correlation between seed colour and total seed flavonoid content in 166 DH lines from the cross Express 617 (black seed) x 1012-98 (yellow seed). The correlation is very weak, suggesting that a significant proportion of the total flavonoids are composed of colourless compounds that have no effect on seed colour.

Preliminary localisation of significant QTL for total flavonoids and individual flavonoid compounds indicated that some loci co-localise with major QTL for seed colour that were described previously by Badani et al. (2006). On the other hand numerous QTL were detected that do not appear to have a significant effect on seed colour but may represent epistatic gene loci that contribute to the high environmental variation seen in yellow- and brown-seeded *B. napus* lines. By comparing the levels of flavonoid compounds with QTL it may be possible to identify genes within the flavonoid biosynthesis pathway

(Routaboul et al. 2006, Lepiniec et al. 2006; Figure 3) that are responsible for minor differences in seed colour in *B. napus*.

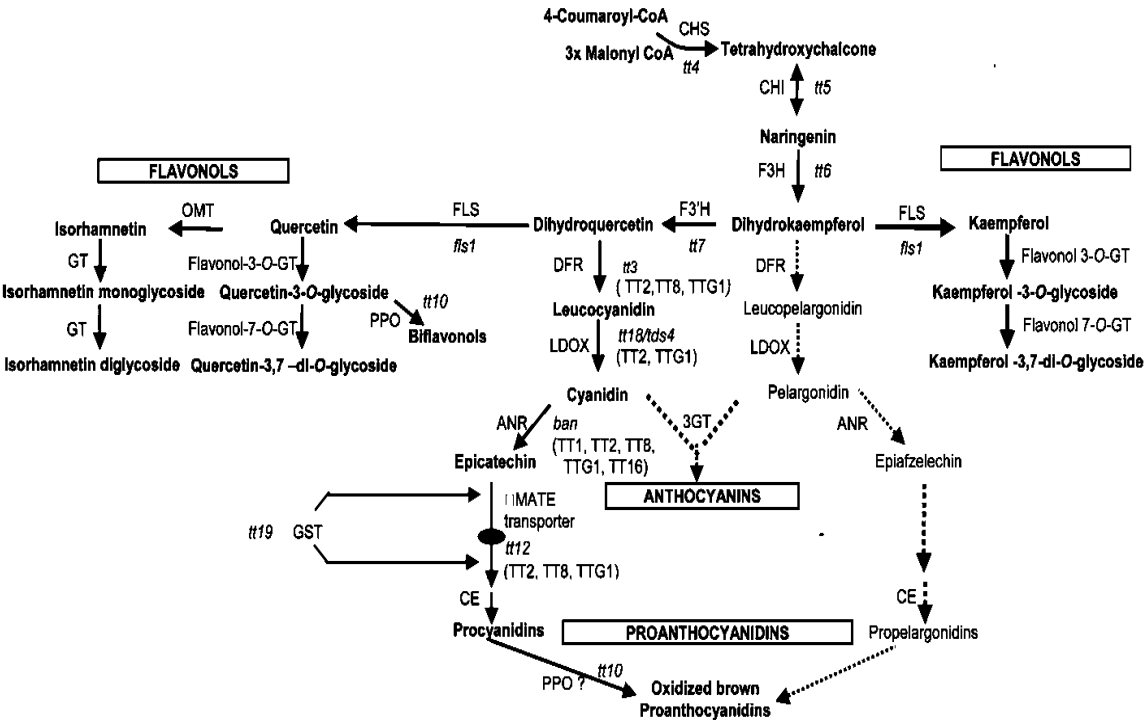


Figure 3: The flavonoid biosynthesis pathway of Arabidopsis, including key structural and regulatory *transparent testa* (TT) genes. Detailed knowledge of flavonoid biosynthesis can help to identify genes involved in differences observed in flavonoid composition between yellow- and black-seeded oilseed rape lines. In particular, the lack of epicatechin in yellow-seeded lines may point to mutations in structural or regulatory genes directly involved in epicatechin accumulation. Diagram from Routaboul et al. (2006).

Discussion

Mapping of candidate genes for flavonoid biosynthesis and comparison to QTL for major flavonoid compounds will enable us to identify genes involved in the expression and control of seed colour in yellow-seeded oilseed rape. Mapping of orthologous copies for relevant Arabidopsis *transparent testa* genes, with the help of synteny-based and map-based cloning techniques, is currently in progress to help achieve this aim.

Acknowledgements

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References

Badani AG, RJ Snowdon, R Baetzel, FD Lipsa, B Wittkop, R. Horn, A De Haro, R Font, W Lühs, W Friedt (2006) Co-localisation of a partially dominant gene for yellow seed colour with a major QTL influencing acid detergent fibre (ADF) content in different crosses of oilseed rape (*Brassica napus*). Genome (in press)

Lepiniec L, Debeaujon I, Routaboul JM, Baudry A, Pourcel L, Nesi N, Caboche M (2006) Genetics and biochemistry of seed flavonoids. Annu Rev Plant Biol 57: 405-430

Routaboul JM, Kerhoas L, Debeaujon I, Pourcel L, Caboche M, Einhorn J, Lepiniec L (2006) Flavonoid diversity and biosynthesis in seed of *Arabidopsis thaliana*. Planta, 224: 96-107

Canola protein hydrolyzates

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Abstract

Canola protein hydrolyzates were prepared using commercial enzymes, namely Alcalase and Flavrouzyme. While Alcalase is an endopeptidase, Flavourzyme acts as both endo- and exopeptidase. The hydrolyzate production was carried out under pre-selected conditions to reach a desired degree of hydrolysis (DH). The hydrolyzates so prepared were effective as antioxidants in inhibiting oxidation in model systems, mainly by scavenging of free radicals, presumably via participation of hydroxyl groups of aromatic amino acids as hydrogen donors. This effect was concentration-dependent and was also influenced by the type of enzyme employed in the process. The hydrolyzates were found effective in enhancing water-holding capacity and cooking yield in a meat model system. Canola hydrolyzates are therefore useful in terms of their functionality and as functional food ingredients.

Key words: Canola, protein hydrolyzates, Alcalase, Flavourzyme, antioxidant, water-holding capacity

Introduction

Canola is a trademarked cultivar of the rapeseed family, one of the top five oilseed crops grown around the world. It has a low content of both glucosinolates and erucic acid. Canola plant is one of the major crops in the agricultural industry of North America, especially in Canada. Considerable quantities of canola are cultivated in Canada and the seed and oil are exported to different parts of the world, including the United States, Mexico, Japan, China and Europe. Canola oil, as a widely consumed edible oil, is produced from canola seeds. The oil is extracted from seeds in large scale leaving behind a bulk of protein-rich meal as the byproduct. Utilization of canola protein following oil extraction has been a subject of continued interest.

The defatted canola meal is composed mainly of protein and is considered a potential source of protein for animal feed and fertilizer as well as for human consumption. However, the insolubility of both crude canola meal after oil extraction and its protein isolates has always been a challenge for their application in the food and animal feed industries. Investigations have been carried out in order to modify the physical and chemical characteristics of canola proteins without altering their biological and functional properties. Hydrolyzing the peptide bonds in the proteins by chemical or enzymatic means yields free amino acids and short-chain polypeptides and has been found to be an efficient and cost effective way to enhance the solubility and thus improving the utilization of proteins from canola meal. Protein hydrolyzates as a value-added product from different sources have been studied. These include capelin protein hydrolyzates (Shahidi *et al.,* 1995), seal protein hydrolyzates (Shahidi *et al.,* 1994), casein protein hydrolyzates (Mahmoud *et al.,* 1992), whey protein hydrolyzates (Turgeon *et al.,* 1992), rice bran protein hydrolyzates (Hamada, 2000) and sunflower seed protein hydrolyzates (Conde *et al.,* 2005), among others. The process of hydrolysis is able to increase the solubility of proteins to various extents, depending on the protein composition and the degree of hydrolysis. Moreover, the protein hydrolyzates produced may possess some physicochemical characteristics and bioactivities that are not found in the original proteins, such as water-holding capacity and antioxidant activity. Shahidi *et al.* (1995) reported that capelin protein hydrolyzates at a level of 0.5-3.0% inhibited the formation of thiobarbituric acid reactive substances (TBARS) by 17.7-60.4%. A similar effect was documented for potato protein hydrolyzates (Wang and Xiong, 2005). However, canola protein hydrolyzates as a potential antoxidant has not been studied. This work evaluated the antioxidant activity of canola protein hydrolyzates prepared by two different proteases, namely, Alcalase, an endopeptidase and Flavourzyme, a mixture of endopeptidase and exopeptidase. Their water-holding capacity in a meat model system during cooking was also examined.

Material and Methods

Preparation of canola protein hydrolyzates: Whole canola seeds were ground and defatted using hexane as the extraction solvent. The defatted canola meal samples were vacuum-packed and stored at -20ºC prior to hydrolysis. The crude protein content in the meal was determined by Kjeldahl analysis according to the AOAC (1990) in order to calculate the enzyme to protein ratio for the hydrolysis. The meal samples were divided into 3 groups and hydrolyzed under pre-selected conditions. Sample 1 was hydrolyzed at 50 ºC and pH 8 for 1 h using Alcalase and sample 2 at 50ºC and pH 7 for 2 h using Flavourzyme; sample 3 used the combination of the two enzymes, i.e. hydrolyzing with Alcalase for 1 h followed by Flavourzyme for an additional 2 h. Conditions were constantly monitored and maintained throughout the process. The pH value, which changes as a result of hydrolysis, was kept constant by addition of a known amount of sodium hydroxide. Upon completion of the hydrolysis, the enzymes were deactivated by dropping the pH to 5. The reaction mixtures were then filtered and hydrolyzates collected. The protein hydrolyzates obtained were freeze-dried and stored at -20 ºC for subsequent analyses.

DPPH radical scavenging assay: The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of enzymatically prepared canola protein hydrolyzates were determined following the procedure described by Shahidi *et al.*

(2006) with minor modifications. Hydrolyzate samples at different concentrations (1.25, 2.5, 5 and 10 mg/ml) were mixed with 50 μM ethanolic DPPH solution and the mixtures were allowed to stand at room temperature for 30 min. The absorbance was then read at 517 nm using a spectrophotometer and the scavenging of DPPH by protein hydrolyzates was calculated as follows:

$$\% \text{ scavenging} = 100 \text{ X } [Abs_{control}-(Abs_{sample}- Abs_{blank})]/Abs_{control,}$$

where, $Abs_{control}$ stands for absorbance of DPPH without protein hydrolyzates, while Abs_{blank} represents absorbance of protein hydrolyzates without DPPH.

Determination of reducing power: Reducing power of canola protein hydrolyzates was measured according to Duh *et al.* (2001) with some modifications. Briefly, canola protein hydrolyzates were dissolved in a 0.2M phosphate buffer solution (pH 6.6) at concentrations of 1.25, 2.5, 5 and 10 mg/ml. They were then added to a 10 mg/ml potassium ferricyanide solution and incubated at 50ºC for 20 min. To the mixtures after incubation, deionized water and a ferric chloride solution (1.0 mg/ml) were added. The absorbance was recorded immediately at 700 nm. A control with no hydrolyzates present and a blank containing only hydrolyzate samples were used because proteins also absorb at the same wavelength.

Determination of water-holding capacity: Water-holding capacity of canola protein hydrolyzates in a meat model system was determined according to Shahidi and Synowiecki (1997). To a mixture containing 8.5 g of ground pork and 1.5 g of distilled water, the canola protein hydrolyzate samples were added at concentrations of 0.5 and 1% (w/w) and mixed thoroughly. The mixture was allowed to stand in a cold room for 1 h and subsequently cooked at 95ºC in a water bath for 1 h followed by cooling under a stream of cold tap water. The drip water was removed with a filter paper and the weight of the meat was recorded. The drip volume was obtained by calculating the weight loss after cooking.

Results

Based on the amount of sodium hydroxide consumed over the course of the reaction, the sample hydrolyzed with Alcalase and with the combination of Alcalase and Flavourzyme had similar degrees of hydrolysis (DH), while the lowest DH was observed in Flavourzyme hydrolyzates (data not shown).

The results of the DPPH assay and the reducing power test show that all protein hydrolyzates possessed antioxidant properties. Figure 1 presents the DPPH radical scavenging capacity of canola protein hydrolyzates prepared by different enzymes. A concentration-dependent effect was observed for all protein hydrolyzates on scavenging of DPPH radicals. The hydrolyzates generated by Alcalase alone and those generated by the combination of Alcalase and Flavourzyme appeared to have similar scavenging capacity against DPPH radicals. The hydrolyzates prepared by Flavourzyme alone showed the highest antioxidant activity among all samples at all concentrations.

A similar trend was obtained for reducing power. The reducing power of the hydrolyzates increased with increasing concentration (Figure 2). The results strongly correlated with those of DPPH scavenging capacity. The Flavourzyme hydrolyzates had the highest reducing power among all samples, while hydrolyzates by Alcalase alone and by combination of Alcalase and Flavourzyme were not significantly different in their reduction potentials.

The canola protein hydrolyzates enhanced water-holding capacity of meat during cooking and thus improved the cooking yield. The drip volume decreased in meat treated with protein hydrolyzates in comparison with that devoid of protein hydrolyzates. This effect was concentration-dependent and influenced by the enzymes employed during hydrolysis (Figure 3). Flavourzyme hydrolyzates were most effective in decreasing the drip volume, followed by hydrolyzates prepared by combination of Alcalase and Flavourzyme and then those by Alcalase alone.

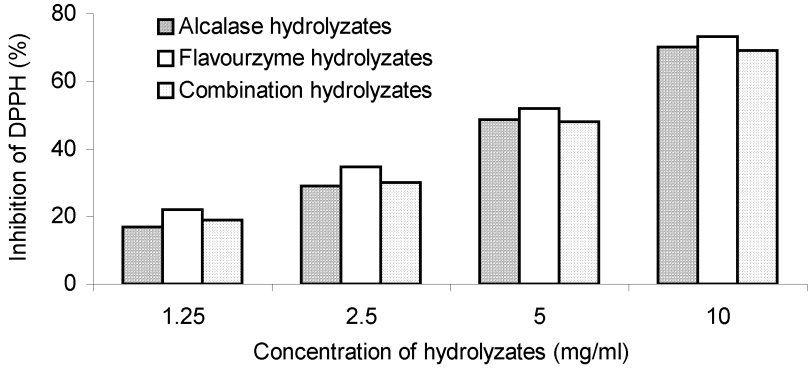

Fig. 1. DPPH radical scavenging capacity of canola protein hydrolyzates

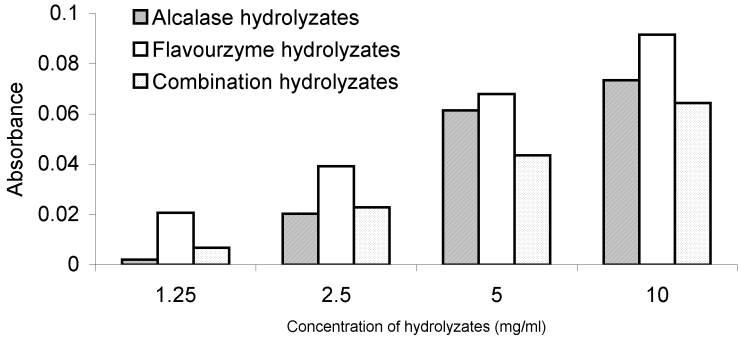

Fig. 2. Reducing power of canola protein hydrolyzates

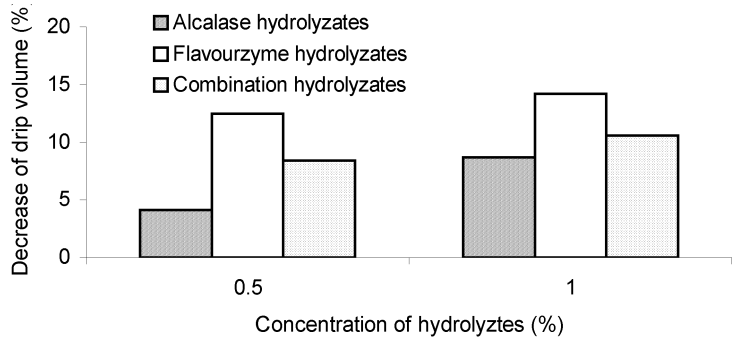

Fig. 3. Decrease of drip volume (%) in meat treated with canola protein hydrolyzates

Discussion

The enzymes employed in this work hydrolyzed the canola meal proteins to different degrees. The lowest DH was found in Flavourzyme hydrolyzates, while unexpectedly, Alcalase and the combination of Alcalase and Flavourzyme resulted in similar DH. It is possible that in the combination, the Flavourzyme did not hydrolyze or hydrolyzed only to a limited extent the proteins and therefore most of the hydrolysis was carried out by Alcalase. In addition to varied DH, the enzymes hydrolyze proteins in different manners, and as a result generate protein hydrolyzates with varied compositions. Alcalase as an endopeptidase cleaves peptide bonds in the interior of the polypeptide chain, producing mainly small portions of proteins or large polypeptides and great portions of small- and medium-size peptide fractions (Adler-Nissen, 1986). The Flavourzyme hydrolyzates generally contain more low-molecular-weight components than Alcalase hydrolyzates, such as small/medium peptides or amino acids, as Flavourzyme acts as both endo- and exopeptidase (Hamada, 2000). The composition of the resultant protein hydrolyzates determines their functional properties and thus their potential applications in the food and animal feed industries.

The canola protein hydrolyzates prepared by both enzymes exhibited antioxidant effectiveness in radical scavenging, presumably via participation of hydroxyl groups of aromatic amino acids as hydrogen donors. Like most other antioxidants, they also showed a reducing power in redox reactions. Their antioxidant activity was concentration-dependent and varied among samples. Flavourzyme hydrolyzates had the highest radical scavenging capacity and reducing power, indicating that the polypeptide fractions obtained by Flavourzyme possessed a higher antioxidant activity than those prepared by Alcalase and the combination of Alcalase and Flavourzyme. The hydrolyzates produced by the combination of two enzymes did not differ significantly from those by Alcalase alone in their antioxidant activity, possibly because Flavourzyme did not contribute to the hydrolysis in combination, as discussed earlier. In addition to their antioxidant activity, all hydrolyzates had a dose-dependent effect in enhancing the water-holding capacity of a meat model system. Their capability in improving the cooking yield of meat was in the order of Flavourzyme hydrolyzates > combination hydrolyzates > Alcalase hydrolyzates, suggesting that smaller-size polypeptides and/or amino acids may be more effective in enhancing water-holding capacity of meat than larger-size polypeptides. Further studies are needed to identify the peptide fractions responsible for antioxidant potential and water-holding capacity of the hydrolyzates.

Conclusions

Canola protein hydrolyzates prepared by commercial proteases Alcalase and Flavourzyme exhibited antioxidant efficacy in terms of radical scavenging capacity and reducing power, with Flavourzyme hydrolyzates possessing the highest antioxidant activity. Alcalase and the combination of Alcalase and Flavourzyme resulted in similar antioxidant activity of the hydrolyzates. The canola protein hydrolyzates were also able to enhance the water-holding capacity of a meat model system and therefore improve the cooking yield.

References

Alder-Nissen, J. (Ed.). (1986). Enzymatic hydrolysis of food proteins. London and New York: Elsevier Applied Science Publishers. P. 110-186.

AOAC (1990). Association of Official Analytical Chemists, Washington, DC.

Conde, J. M., Escobar, M. M. Y., Jiménez, J. J. P., Rodríguez, F. M., Patino, J. M. R. (2005). Effect of enzymatic treatment of extracted sunflower proteins on solubility, amino acid composition, and surface activity. Journal of Agricultural and Food Chemistry 53, 8038-8045.

Duh, P. D., Yen, C. G., Yen, W. J., Chang, L. W. (2001). Antioxidant effects of water extracts from barley (*Hordeum vulgare L.*) prepared under different roasting temperatures. Journal of Agricultural and Food Chemistry 49, 1455-1463.

Hamada, J. S. (2000). Characterization and functional properties of rice bran proteins modified by commercial exoprotease and endoprotease. Journal of Food Science, 65, 305-310.

Mahmoud, M. I., Malone, W. T., Cordle C. T. (1992). Enzymatic hydrolysis of casein: effect of degree of hydrolysis on antigenicity and physical properties. Journal of Food Science 57, 1223-1229.

Shahidi, F., Han, X.-Q., Synowiechi, J. (1995). Production and characteristic of protein hydrolysates from capelin (*Mallotus villosus*). Food Chemistry 53, 285-293.

Shahidi, F., Liyana-Pathirana, C. M., Wall, D. S. (2006). Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chemistry 99, 478-483.

Shahidi, F., Synowecki, J. (1997). Protein hydrolyzates from seal meat as phosphate alternatives in food processing applications. Food Chemistry 60, 29-32.

Shahidi, F., Synowiecki, J., Balejko, J. (1994). Proteolytic hydrolysis of muscle proteins of harp seal (*Phoca groenlandica*). Journal of Agricultural and Food Chemistry 42, 2634-2638.

Turgeon, S. L., Gauthier, S. F., Paquin, P. (1992). Emulsifying property of whey peptide fractions as a function of pH and ionic strength. Journal of Food Science 57, 601-604.

Wang, L. L., Xiong, Y. L. (2005). Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. Journal of Agricultural and Food Science 53, 9186-9192.

A new method for preparation of non-toxic, functional protein hydrolysate from commercial mustard cake

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Abstract

Rapeseed/mustard is an important oilseed crop and ranks second in the world production of oilseed after soybean and second major oilseed crop in India. Traditionally, most of the mustard seeds are crushed for oil production and the cake obtained contains 34-40% protein, being used for animal feed or fertilizer because of the presence of glucosinolates, phytates, phenolics and fiber. In the present study, the defatted commercial mustard cake was fractionated to obtain low hull and high protein fraction. The fractionation reduced the fiber content by 60%. The low hull high protein fraction was used in the preparation of protein hydrolysate. The process comprises extraction of the protein in 0.1 M NaCl containing 0.1% ascorbic acid, incubation, extraction at optimum pH and treating with adsorbent.The protein was thermally coagulated, separated, washed and dissolved in water and treated with enzyme alkalase and spray dried. The yield of protein hydrolysate was 60% with a protein content of 80%. The protein hydrolysate had a very low content of isothiocyanate (0.12 mg/g) and oxazolidinethiones (0.09 mg/g) compared to the starting material (1.69 mg/g and 2.08 mg/g respectively). The reduction in isothiocyanate and oxazolidinethiones was 98.6 and 99.1%, respectively. The process resulted in the reduction of the phytate and phenolics by 98 and 97.5%, respectively and trypsin inhibitor activity totally inactivated. Nutritional evaluation indicated that the protein hydrolysate had better nutritional characteristics compared to starting material. Protein hydrolysate showed good functional properties with a solubility of more than 50% in all pH values.

Key worlds: Mustard cake, Protein hydrolysate

Introduction

Mustard/rapeseed is one of the major oilseed crops of India. Most of the seeds are used for oil extraction in Ghanies or expellers. In the traditional processing of mustard/rapeseed, the material is crushed without dehulling. The hull imparts dark color and contributes high amount of crude fiber (27%) in the meal. The oil obtained is dark in color and is not acceptable. The dark color cake finds very limited use in the food/feed purposes (Bell, 1984). The presence of toxic and antinutritional constituents such as glucosinolates, phytates, phenolics and hulls limits the use of rapeseed/mustard as a source of protein in food products. The glucosinolates are hydrolysed by the endogenous enzyme myrosinase to various toxic compounds that interfere with thyroid function and cause liver and kidney damage (VanEtten, Daxenbicher, 1977). Phytates are strong chelating agents that bind to polyvalent metal ions in the body including iron, calcium, magnesium and zinc rendering them unavailable for metabolism (Rutkowski 1971). Phenolic compounds impart bitter taste and dark color to the protein and its products. Tannins are the polyphenolics that complex with proteins suppressing the availability of essential amino acids (Sosulki, 1979). The use of rapeseed/mustard meal is limited in the diets of monogastric animals due to high content of indigestible fiber (Slominski, Campbell, & Guenter, 1994). Several detoxification methods including steaming, toasting, wet heating, water washing, microbial degradation and chemical treatment have been reported in the literature (Woyewoda, & Nakai & Watson; 1978; Maheswari, Stanley, & Gray, 1981). Membrane processing, dialysis, ultrafiltration, diafiltration, ion-exchange and protein micellar mass (PMM) methods for the preparation of protein isolates free of glucosinolates and low in phytates, phenolics and fiber have been reported (Tzeng et al., 1988b; Diosady, Tzeng, & Rubin, 1984; Tzeng, Diosady, & Rubin, 1988a; Tzeng, Diosady, & Rubin, 1990). The recovery of protein by aqueous extraction followed by isoelectric precipitation was low because of multiple isoelectric points. (El-Nockrashy, Mukherjee, & Mangold, 1977; Gururaj-Rao, Kantharaj-Urs, & Narasinga-Rao, 1978; Aruna & Appu-Rao, 1988). Recently, in our laboratory a process for the production of mustard protein isolate, with reduced toxic and antinutritional constituents for food and feed purposes, has been developed and patented (Alireza-Sadeghi, Appu-Rao, & Bhagya, 2004; Alireza-Sadeghi, Appu-Rao, & Bhagya, 2006). The use of heat adversely affects the functional properties of protein, therefore enzyme hydrolysis was used to improve the functional properties of protein.

Material and Methods

Protein hydrolystae was prepared according to the method developed and standardized in our laboratory (Alireza-Sadeghi, Appu-Rao, & Bhagya, 2006) with some modification to include enzyme hydrolysis step. Defatted commercial cake was fractionated to obtain three fraction with different hull contents, The low hull fraction was used for experiment. The low hull fraction of cake was dispersed in 0.1M NaCl containing 0.1% w/v ascorbic acid, in a ratio of 1:15 (w/v) and incubated at 37 ^{0}C for 30min. Then the pH was adjusted to 11 with addition of 1N NaOH. The dispersion was

subjected to shaking for 30 min at room temperature before centrifuging at 5000 rpm for 20 min. The pH of the supernatant was readjusted to 7.0 with 1N HCl. Activated carbon granules (2% w/v) were added and kept for shaking for 1h and filtered. Live steam was injected to the supernatant to raise the temperature to 93 ± 2°C for a period of 10 minatant was readjusted to 7.0 with 1N HCl. Activated carbon granules ()g for 30 min at room temperature before centr, cooled and centrifuged at 5000 rpm to separate protein. The coagulated protein after washing was dispersed in water to a solid content of 20% and adjusted to pH 8. Enzyme Alcalase (1ml/100g dry protein) was added and incubated at 50°C for 1h. After hydrolysis, the temperature was raised to 85°C for 10 min to inactivate enzyme, the solution was spray dried to obtain protein hydrolysate. The protein hydrolysate was analyzed for the presence of antinutritional factors and removal of them was calculated. The nutritional and functional properties of protein hydrolysates were calculated using appropriate methods.

Results

The chemical composition of whole seed, cake and its fractions are given in Table 1. The oil extraction by Ghani pressing increased the protein and crude fiber contents of cake from 25 to 32% and 11 to 14% respectively, compared to the starting seeds. The cake obtained after Ghani pressing still contained around 13% oil and was recovered by solvent extraction to obtain a defatted cake with low oil content. Removal of oil was beneficial during separation into three different fractions. The chemical composition of the fine and coarse fractions didn't show any significant differences (Table 1). These two fractions were combined and named as **"low hull high protein fraction"**.

Table 1. Chemical Composition of Mustard Seed, Cake and its Fractions

Constituents	Whole seed (Commercial Variety)	Cae	Low Hull Fine Fraction	Low Hull Coarse Fraction	High Hull Fraction
Moisture	6.3 ± 0.3	11.2 ± 0.2	8.8 ± 0.2	9 ± 0.1	9 ± 0.3
Protein (N × 6.25)	24.8 ± 0.4	32.3 ± 0.8	40.8 ± 0.2	40.6 ± 0.2	30.5 ± 0.5
Ash	3.7 ± 0.1	5.4 ± 0.2	6 ± 0.15	6.2 ± 0.1	5.5 ± 0.2
Fat	37.7 ± 0.1	13.2 ± 0.3	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
Crude Fiber	11 ± 0.5	14 ± 0.5	5.3 ± 0.2	5.5 ± 0.1	17 ± 0.5
Carbohydrate (by diff.)	16.5 ± 0.3	23.9 ± 0.5	36.9 ± 0.3	36.5 ± 0.2	35.8 ± 0.4

The high hull fraction contained low protein and higher amount of crude fiber (17%). Separation of hull increased the protein content and reduced the crude fiber content. The results showed that defatting and fractionation reduced the amount of crude fiber by 60 % and increased the amount of protein by 28 % compared to the starting cake.

The anti-nutritional factors presented in low hull fraction and protein hydrolysate are presented in Table 2. The content of isothiocyanate and oxazolidinethione in low hull fraction was 1.69 and 2.08 mg/g and it reduced to 0.12 and 0.09 mg/g in protein hydrolysate, respectively. The reduction achieved was around 99% as compared to low hull fraction of cake. The phenolics and phytic acid content of low hull fraction were 1.8 and 5.3%, respectively. In the protein hydrolysate, they were reduced to 0.15 and 0.35%, respectively. The reduction of these constituents was around 98% on the basis of protein yield. The trypsin inhibitor activity in low hull fraction was 3.8 TIU/mg of protein and completely inactivated in protein hydrolysates.

Table 2-Anti-nutritional Factors Present in Low Hull Fraction of

Constituents	Low Hull Fraction of Cake	Protein hydrolysate	Removal (%)
Isothiocyanates (mg/g)	1.69 ± 0.7	0.12 ± 0.01	98.6 ± 0.1
5-Vinyloxazolidine-2-thione (mg/g)	2.08 ± 0.04	0.09 ± 0.005	99.1 ± 0.1
Phenolics (%)	1.8 ± 0.05	0.15 ± 0.02	97.5 ± 0.2
Phytic Acid (%)	5.3 ± 0.08	0.35 ± 0.05	98 ± 0.2
Trypsin Inhibitor activity (TIU/mg protein)	3.5 ± 0.1	ND	100

Table 3.Nutritional Indices of Low Hull Fraction of Cake and Protein Hydrolysate

Parameters		Low Hull Fraction of Cake	Protein Hydrolysate from Cake
In vitro protein digestibility (%)*		80.6 ± 0.5	94.8 ± 0.2
C-PER		2.1	2.46
Essential Amino Acid Index		80.9	85.7
Predicted Biological Value		76.5	81.7
Nutritional Index		33.2	65.1
PDCASS	2-5 years old	68.8	69.2
	10-12 years old	90	91
	Adults	100	100
Available lysine (%)		4.45 ± 0.05	3.75 ± 0.05

Cake and Protein hydrolysate

The calculated nutritional indices of low hull fraction of cake and protein hydrolysate are presented in table 3. The increased i*n vitro* digestibility may be due to enzyme hydrolysis of proteins. All nutritional indices were higher in protein hydrolysate compared to low hull fraction of cake indicating higher nutritional value of protein hydrolysate.

Figure 1 show the protein solubility profile of cake protein hydrolysate (DH 8.7 ± 0.15) compared to protein concentrate and low hull fraction of cake at different pH values. The protein concentrate prepared from low hull fraction of cake (without using enzyme hydrolysis) had a solubility of 20-35% which increased to 50-65% on hydrolysis.

Table 2 shows the functional properties of low hull fraction of cake, protein concentrate and hydrolysate. In all cases, except foam stability, other functional properties improve due to protein hydrolysis compared to protein concentrate. Apparently, hydrolysate are capable of foaming but lack strength to maintain the foam as a result of reduction in molecular (peptide) size.

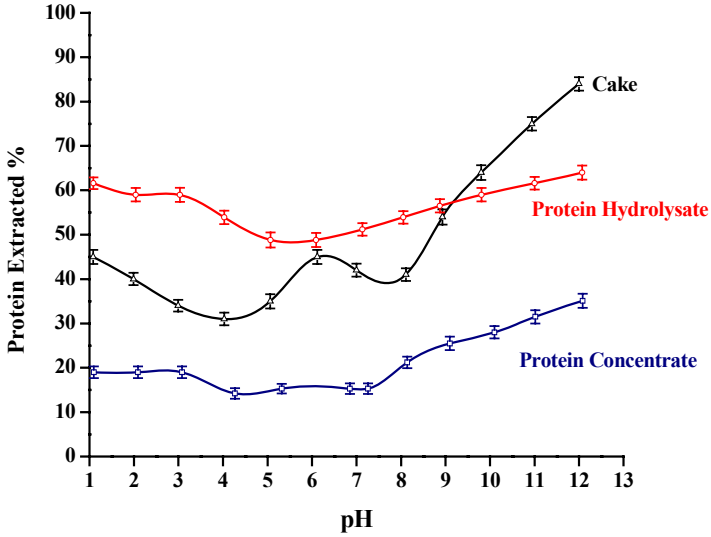

Fig 1. Protein Solubility Profile of Low Hull Fraction of Cake, Protein Concentrate and Hydrolysate

Table 4. Functional properties of cake, protein concentrate and hydrolysate

Sample	Water absorption capacity g/100g	Fat absorption capacity ml/100g	Foam capacity %	Foam stability %	Emulsion capacity ml/g
Low hull fraction of cake	270 ± 5	160 ± 5	90 ± 3	75 ± 5	54 ± 1
Cake protein concentrate	204 ± 3	90 ± 5	55 ± 5	45 ± 3	31 ± 0.5
Cake protein hydrolysate	256 ± 5	132 ± 5	85 ± 5	40 ± 3	40.5 ± 0.5

Discussion

Different step of protein recovery including activated carbon treatment, heat coagulation and washing removes all antinutritional constituents with high yield and a protein hydrolysate with 80% protein content. Enzyme modification improves protein solubility and therefore positively affects other functional properties. The nutritional value expressed by different indices shows a high quality protein which can be used in human diet.

Conclusion

The detoxification process that used in this research removes more than 98 % of all antinutritional factors present in original material. The protein without hydrolysis showed good nutritional values but lack proper functional properties. Enzyme modification by alkalase improved functional properties and protein showed proper functionality for food uses. Therefore incorporation of enzyme modification in extraction method can improve protein functionality for food uses.

References

Bell, J.M. (1984). Nutrient and toxicants in rapeseed meal: a review. J. Anim. Sci., **58,** 996.

VanEtten, C.H., and Daxenbichler, M.E. (1977). Glucosinolates and derives products in cruciferous vegetables: total glucosinolates by retention on anion exchange resin and enzymatic hydrolysis to measure released glucose. J. Assoc. Off. Anal. Chem., **60**, 964.

Rutkowski, A. (1971). The feed value of rapeseed meal. J. Am. Oil Chem. Soc., **48**, 863.

Sosulski, F. (1979). Organoleptic and nutritional effects of phenolic compounds on oilseed protein products: A review. J. Am. Oil Chem. Soc., **56**,711.

Slominski, B.A., Campbell, L.D., and Gunter, W. (1994). Oligosaccharides in canola meal and their effect on non-starch polysaccharide digestibility and true metabolizable energy in poultry. Poultry Sci., **73**, 156.

Woyewoda, A.D., Nakai, S., and Watson, E.L. (1978). Detoxification of rapeseed protein products by activated carbon treatment. Can. Inst. Food Sci. Technol. J., **11**, 107.

Maheshwari, P.N., Stanley, D.W., and Gray, J.I. (1981). Detoxification of rapeseed products. J. Food Protec., **44**, 459.

Tzeng, Y.M., Diosady, L., and Rubin, L. (1988a). Preparation of rapeseed protein isolate using ultrafiltration, precipitation and diafiltration. Can. Inst. Food Sci. Technol. J., **21**, 419.

Tzeng, Y.M., Diosady, L., and Rubin, L. (1988b). Preparation of rapeseed protein isolate by sodium hexametaphosphate extraction, ultrafiltration, diafiltration and ion exchange. J. Food Sci., **53**, 1537.

Tzeng, Y.M., Diosady, L., and Rubin, L. (1990). Production of canola protein materials by alkaline extraction, precipitation and membrane processing. J. Food Sci., **55**, 1147.

Diosady, L.L., Tzeng, Y.M., and Rubin, L.J. (1984). Preparation of rapeseed protein concentrates and isolates using ultrafiltration. J. Food Sci., **49**, 768.

El-Nockrashy, A.S., Mukherjee, K.D., and Mangold, H.K. (1977). Rapeseed protein isolate by countercurrent extraction and isoelectric precipitation. J. Agric. Food Chem., **25**, 193.

Gururaj Rao, A., Kantharaj Urs, M., and Narasinga Rao, M.S. (1978). Studies on the proteins of mustard seed (B. Juncea). Can. Ins. Food Sci. Technol. J., **11**, 155.

Aruna, V., and Appu Rao, A.G. (1988). Isolation and characterization of low molecular weight protein from mustard (B. juncea). J. Agric. Food Chem., **36**, 1150.

Alireza Sadeghi, M., Appu Rao, A.G., and Bhagya, S. (2004). A process for the preparation of mustard protein isolate with reduced anti-nutritional factors. Indian Patent 480/DEL/04.

Alireza Sadeghi, M., Appu Rao, A.G., and Bhagya, S. (2006). Evaluation of mustard (Brassica juncea) protein isolate prepared by steam injection heating for reduction of anti-nutritional factors. Lebensm. Wiss. U. Technol., 39: 911-917.

Pure or blended rapeseed oil intake to reach human alphalinolenic recommendation increase plasma availability and conversion to eicosapentaenoic (EPA)

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Abstract

Epidemiologic studies have shown that cardiovascular disease (CVD) risk increases in subjects with the lowest alpha-linolenic acid (ALA) intake or plasmatic levels. Recommendations propose a mimimum ALA intake of 2g/day: 0.8% total caloric intake (TCI), but the population is generally below 1g/day (0.4%TCI).

Our objective was to study modifications induced by higher levels of ALA intake, in long-term-diet of 22 healthy men (Monks), via consumption of vegetable oils: 30g per day of a blend (Protect) including 58% as rapeseed or pure rapeseed oil (ALA1.5 and 2.7g/d from oil respectively), inducing an increase of ALA-TCI from the basal diet (0.4%) to 0.93% and 1.36% respectively. Except the changes in oil consumption, the subjects did not modify their dietary habits. Plasma fatty acids were analyzed in phospholipids and cholesteryl esters, and compared to those of the basal diet (30g/d of vegetable oil devoided of ALA).

Results show that increasing levels of ALA in the two diets (Protect and Rapeseed) significantly increases the plasma ALA levels by 90% and 200% respectively and is associated with an increased conversion to Long Chains (LCn-3): EPA by 60% and 130% respectively, but not docosahexaenoic acid (DHA). These modifications, associated to the stability of plasma arachidonic acid (AA) levels, induce a reduction of the pro-aggregating ratio AA/EPA. The plasma lipids (TC, LDL, HDL, TG) remained stable.

Simple nutritional modifications introducing rapeseed oil (pure or in blends) in the usual diet thus increase ALA levels to recommended protective values and could provide an easy way to increase the n-3 cardiovascular protection via the plasma increase of both precursor-ALA and LC-EPA and by reducing the pro-aggregating ratio (AA/EPA).

Key words: Rapeseed oil, alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), bioconversion, n-3 fatty acids.

Introduction

Cardiovascular disease (CVD) is the number one public health problem in western countries and epidemiologic studies have shown that the risks of coronary heart disease and stroke occurred in subjects whose levels of alpha-linolenic acid (ALA) intake or plasma ALA levels are the lowest and lower than in healthy subjects (1-3).

Furthermore, the plasma levels of ALA in cholesteryl esters (CE) have been shown to represent a good marker (or factor) of protection against stroke and cardiovascular diseases.

Secondary intervention studies reported that rapeseed oil consumption (ALA rich oil) by increasing plasma ALA levels could be protective against these diseases (*Lyon Study*) (4-5).

In many western countries, the dietary intake of ALA is far below the recommendations of guidelines: the levels of ALA represent only 0.3 to 0.4% of the total caloric intake (TCI) (6-7), while the recommendations require 0.8% at least (2g per day or more) (8).

Our objective was to introduce the consumption of rapeseed blend and pure rapeseed oils, in long-term-diets of a group of healthy human males, to reach ALA intake recommendations and to evaluate the modifications induced on plasma ALA bioavailability and bioconversion to long chain derivatives of n-3 (LC-n-3: eicosapentanoic acid EPA and docosahexaenoic acid DHA).

By using these rapeseed blend or pure oils, the tested diets will allow an enrichment in ALA intake and a simultaneous impoverishment in linoleic acid (LA) resulting in a lower LA/ALA ratio (recommended value of 5) which is also thought to modulate the bioconversion of ALA to LC-n-3: EPA and DHA.

Methods

Diet and study design

The protocole was carried on 22 healthy men living in a monastery (mean age 55 ±20 years). The natural level of oil consumption by the subjects was 30 g/day (sunflower) during the Basal diet and was maintained during the whole protocole, including the consumption of a rapeseed blend (Protect) or pure rapeseed oils for 4 months each. The oils were provided by Lesieur Company.

The **Protect blend** and pure **Rapeseed** oils were both rich in ALA, (5% and 9% respectively). In Rapeseed oil, the level of LA is naturally low (23%) and the oleic acid (OL) level is high (59%). Protect blend was prepared with 58% of rapeseed oil supplemented with 24% of olive oil in order to obtain levels of LA and OL similar to Rapeseed (table). The LA/ALA ratios were drastically decreased: 4.9 for the Protect blend, 2.6 for the Rapeseed compared to the Sunflower oil (devoided of ALA) used in the Basal diet of the Monastry (670).

Since the oil consumption of subjects was maintained at 30 g/day, the ALA intake introduced by Protect blend and Rapeseed oils were respectively 1.49 g/day and 2.7 g/day, compared to the 0.03 g/day of the basal Sunflower oil.

The fatty acid (FA) compositions of the two tested oils were very similar in terms of OL and LA and the major difference was the level of ALA, almost double for Rapeseed oil compared to Protect blend oil.

Table: Fatty acid distribution in vegetable oils

Diets	Basal	Protect	Rapeseed
Fatty acid composition of the different oils			
18:1n-9 (OL)	20.0	57.8	59.0
18:2n-6 (LA)	67.0	24.4	23.0
18:3n-3 (ALA)	0.1	5.0	9.0
18:2n-6/18:3n-3 (LA/ALA)	670.0	4.9	2.6

Except for the changes in oil consumption, the subjects did not modify their dietary habits during the whole protocole: both basal and interventional diets provided roughly 2500 kcal and 15% of energy as proteins, 51% as carbohydrates and 34% as lipids.

The FA composition of the Basal diet was represented by 11% of saturated (30 g/day), 11% of monounsaturated (30 g/day) and 12% of polyunsaturated FA (33 g/day) due to the natural consumption of highly polyunsaturated sunflower oil (30 g/day) in the Monastry at the beginning of the protocole.

Compared to the Basal sunflower diet, the dietary modifications resulted in an increase of ALA intake during the Protect and Rapeseed diets (+136% and +245% respectively, fig. 1A), an increase of OL (+50%) and a decrease of LA (-50%) intakes for both Protect and Rapeseed diets (fig. 1B). In terms of total caloric intake (TCI), the ALA intake of Protect and Rapeseed diets represented respectively 0.9% and 1.4% of TCI (higher than the Basal diet and the recommended values: 0.4% and 0.8% respectively). The LA/ALA ratio was reduced within the recommended values, from 5.4 to 3.6, which is far below the value of Basal sunflower diet (23.8, fig. 1C).

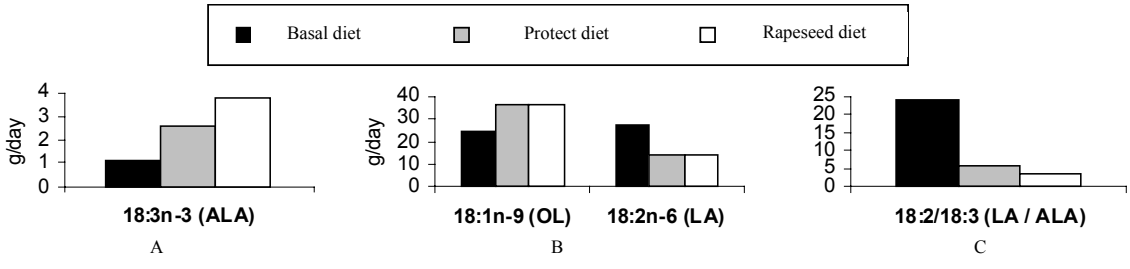

Fig. 1: Quantities of ALA (A), OL and LA (B) and LA/ALA ratios (C) in the dietary intake

Plasma lipid analysis

The impact of these diets on lipids (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol) was assessed by enzymatic commercial kits.

The plasma fatty acids (FA) were analyzed in phospholipids (PL) and cholesterol esters (CE) by gas-liquid chromatography, and compared to those of the Basal diet.

Statistics

Statistical analysis were performed by ANOVA, followed by student's t-test. Different letters indicate significant differences between diets ($p < 0.05$).

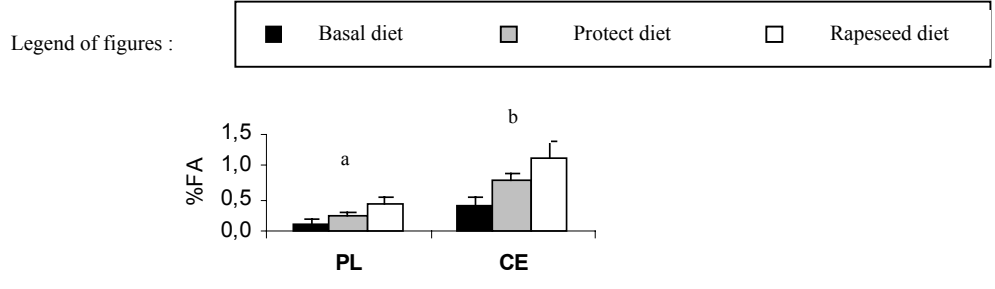

Fig. 2: Plasma levels of 18:3 n-3 (ALA)

Results

The plasma lipids (Total Cholesterol, LDL-C, HDL-C and Triglycerides) remained stable whatever the diet consumed (data not shown).

Compliance

Compliance of the subjects with the diets was verified by the evaluation of the plasma FA modifications resulting of the dietary FA changes introduced by the protocole: plasma OL increased and LA decreased significantly with the two interventional diets (data not shown). Thus, plasma FA modifications corresponded to the FA changes of the dietary protocole.

Bioavailability and bioconversion of ALA to LCn-3

FA of plasma PL and CE showed that raising the levels of ALA with Protect and Rapeseed diets significantly increased the plasma ALA levels by around 90% and 200% respectively (fig. 2).

Higher levels of plasma ALA obtained with Protect and Rapeseed diets compared to Basal diet were associated with an increased conversion to EPA by 60% and 130% respectively (fig. 3A). No significant modification of DHA levels was observed (fig. 3B).

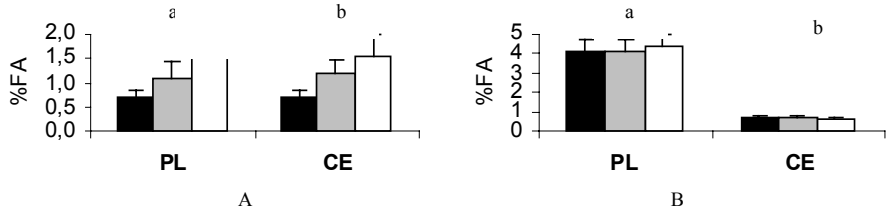

Fig. 3: Plasma levels of 20:5 n-3 (EPA),(A), and 22:6n-3 (DHA), (B).

Plasma ratios: AA/EPA and LA/ALA

Despite the decrease in LA observed in plasma PL (-10% to -15%) with Protect and Rapeseed diets, the levels of arachidonic acid (20:4n-6, AA) were not significantly modified (data not shown). However, a dramatic significant reduction of the pro-aggregant 20:4n-6/20:5n-3 ratio (AA/EPA), due to the increase of EPA, was obtained: -38% and -61% in plasma PL with Protect and Rapeseed diets respectively (fig. 4A). Compared to the Basal diet, the ratio of precursors, LA/ALA, was also significantly reduced in plasma PL with both diets: by half with Protect and by four with Rapeseed (fig. 4B)

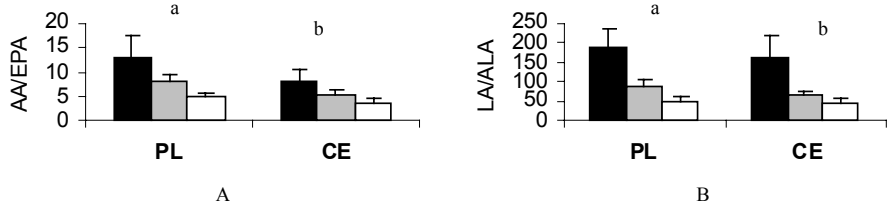

Fig. 4: Plasma levels of AA/EPA (A), and LA/ALA (B).

Individual variations of plasma FA

Analysis of individual variations of plasma FA showed clearly the benefit of the two interventional diets enriched in ALA on the plasma levels of ALA and EPA (fig.5A-C).

All subjects at the end of Protect and Rapeseed diets presented ALA levels in plasma CE above the values of 0.6% and 0.8% respectively (fig.5A), values which had been proposed for protection against stroke and cardiovascular diseases.

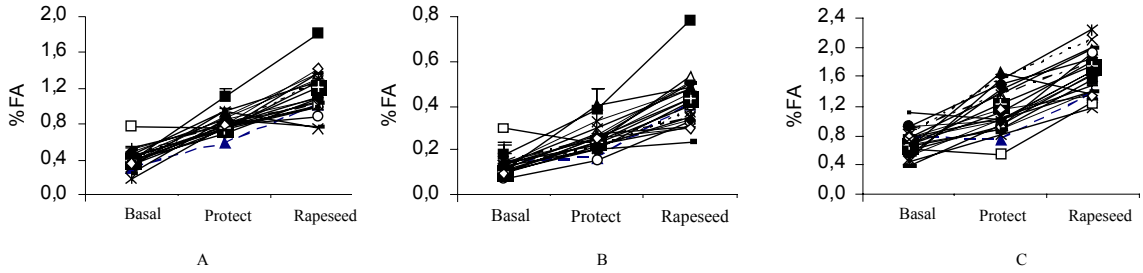

Fig. 5: Individual variations of plasma fatty acids

(A) ALA in plasma CE, (B) ALA in plasma PL, (C) EPA in plasma PL

Conclusions

Simple nutritional modifications, introducing rapeseed oil (pure or in blend) in the usual diet, increase ALA intakes to recommended protective values and could provide an easy way to increase the n-3 cardiovascular protection via the plasma increase of both ALA and LC-EPA and by reducing the pro-aggregant ratio (AA/EPA).

References

1. Sandker GW., Kromhout D., Aravanis C., Bloemberg BP., Mensik RF. et al. (1993). Serum cholesteryl ester fatty acids and their relation with serum lipids in elderly men in Crete and The Netherlands. Eur J Clin Nutr, 47, 201-8.
2. Simon JA., Fong J., Bernert JT. Jr., Browner WS. (1995). Serum fatty acids and risk of stroke. Stroke, 26, 778-82.
3. Erkkila AT, Lehto S, Pyorala K, Uusitupa MI. (2003). Euroaspire. N-3 fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. Am J Clin Nutr,78, 65-71
4. De Lorgeril M., Renaud S., Mamelle N., Salen P., Martin JL., Monjaud I., Guidollet J., Touboul P., Delaye J. (1994). Mediterranean alphalinoleic acid-rich diet in secondary prevention of coronary heart disease. Lancet, 343, 1454-59.
5. Singh RB., Dubnov G., Niaz MA., Ghosh S., Singh R., Rastogi SS., Manor O., Pella D., Berry EM. (2002). Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial. Lancet, 360 (9344), 1455-61
6. Combe N., Boué C. (2001). Apports alimentaires en acides linoléique et alpha-linolénique d'une population d'Aquitaine. OCL, 8, 118-121.
7. Astorg P., Arnault N., Czernichow S, Noisette N, Galan P, Hercberg S. (2004). Dietary intakes and food sources of n-6 and n-3 PUFA in French adult men and women.Lipids, 39(6), 527-35.
8. AFSSA. (2003). Goupe de travail sur les omega3. Acides gras de la famille omega3 et systeme cardiovasculaire : interet nutritionnel et allegations.

Study on extraction, isolation and bioactivities of phytosterol from rapeseed

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Abstract

In this paper, the research results that were carried out in our laboratory have been reported about the composition, extraction, isolation and structure of phytosterol from deodorizer distillates of rapeseed in our country. The preparation of phytosterol ester that is the derivant of phytosterol has also been covered in this paper. Furthermore, the results about the bioactivities of phytosterol and phytosterol ester, such as antioxidant, serum lipid-lowering, inhibiting tumor and immune enhancement, have also been reported in this paper. These research results provided reliable and theoretical basis for the preparation process of phytosterol and phytosterol ester from deodorizer distillates of rapeseed, and also for the comprehensive utilizations of products with high additional value.

Key words: Rapeseed, Deodorizer distillates, Phytosterol, Phytosterol ester, Bioactivities

1. Introduction

Phytosterol is a kind of natural substance which structure is similar to cholosterol. It is mainly composed of ß-sitosterol, stigmasterol, brassicasterol and campesterol. The study on phytosterol has attracted many research interests in the word because phytosterol bears several functions, such as high safety, inhibiting the absorption of cholesterol and preventing from coronary atherosclerosis. The main method to obtain phytosterol is extracting from deodorizer distillates of vegetable oil when oil was refined. And phytosterol product can be widely used in medicine, foods, cosmetic and feeds. In our laboratory, we have studied the composition, extraction, isolation and structure of phytosterol from deodorizer distillates of rapeseed, studied the preparation of phytosterol ester that is the derivant of phytosterol. The bioactivities of phytosterol and phytosterol ester, such as antioxidant, serum lipid-lowering, inhibiting tumor and immune enhancement were also reported through our research. The application of plytosterols ester was also discussed in this paper.

2. The analytical method of phytosterol ester and its composition

Gas-chromatography(GC) method for the quantitative determination of phytosterols was developed. Gas-chromatography separate condition were selected as follows: HP-5 capillary gas-chromatography column; flame ionization detector(FID); injector temperature 300ºC; oven temperature 285ºC; squalane as internal standard. The method has the advantages of exact and quick.

The determination method of phytosterols ester was established. Methanol sodium as catalyzer, phytosterols ester sample were interesterificated with methanol. The compose and relatively content of fatty acid methyl ester were determinated by GC, accordingly, species and content of fatty acid could be determined. Phytosterols ester sample were saponificated in KOH-alcohol solution, reaction temperature 80ºC, reaction time 2h. Phytosterols ester was translated into free phytosterols. Then, through determining the content of free phytosterols by GC, make a conversion to the content of corresponding phytosterols ester respectively.

UV spectrum, IR spectrum and GC-MS were used to determine the structure of the phytosterols and phytosterols ester. GC-MS crack law of phytosterols and phytosterols ester were summarized.

3. Study on preparation of plytosterols ester

Using pyridine as catalyst, phytosterols acetate (PSA) was synthesized by direct esterification of phytosterols and acetic anhydride. The effect of factors on the yield was studied. The optimal synthetic conditions were as follows: molar ratio of phytosterols to acetic anhydride to pyridine was above 1:9:5; Reaction temperature was 85ºC and reaction time was 2h. The recrystallization method phytosterols acetate were researched. Results of GC analysis of the product show that the purity of product was above 98%.

Phytosterols oletate(PSO) were synthesized by direct esterification of phytosterols and oleic acid. The optimal synthetic conditions obtained were as follows: using sodium bisulfate as catalyst(2.0% molar of phytosterols); molar ratio of oleicacid to phytosterols was 1.3, reaction temperature was 135ºC and reaction time was 8h. The yield of esterify reached 84.3%, the purity of product was above 90%.

Phytosterols stearic (PSS) were synthesized by direct esterification of phytosterols and stearic acid. Through factorial design, the optimal synthetic conditions were obtained as follows: molar ratio of phytosterols to stearic acid was above 1:1.2; reaction temperature was 135ºC and reaction time was 7h. The yield of esterification was 87.8%. Recrystallization method of

phytosterols stearic were researched, the purity of product was above 95%.

4.The antioxidant experiment of phytosterol in lard

The experiments adopted different dosage of phytosterol and different synergism to study the antioxidantive of phytosterol in lard. The results of experiment showed that phytosterol had strong antioxidantive activity in lard, which was concentration dependent. EDTA was the best synergism, next was vitamine E, vitamine C and citric acid.

5.Study on serum lipid-lowering bioactivities of plytosterols ester

Study on the preventive and therapeutic effects of three phytosterol esters (PSA, PSO, PSS) on diet-induced hyperlipidemia in mice and their structure-activity relationships. On preventive experiments, forty healthy, male Kunming mice were randomly divided into five groups on body weight, eight mice per group. The control groups were fed with normal weight, while hyperlipidemic group were fed with the high-cholesterol diet. The rest three groups were given the high-cholesterol diets. At the same time, they were respectively given with three phytosterol esters at the level of 100mg/kg body weight. The effect of phytosterol ester on lipid metabolism in mice was studied. The experiment lasted twenty-eight days. Results showed that compared with the hyperlipidemic group, phytosterol ester could significantly lower the cholesterol levels of mice, including the serum TC, LDL-C and arteriosclerosis index (AI). They also could lower the liver weight, TC and TG. PSA and PSO had ideal preventive effects on diet-induced hyperlipidemia in mice and better than PSS.

6.Study on effect of inhibiting tumor and function of immune enhancement of plytosterols ester

Investigated the anti-tumor effect of Phytosterol Acetate(PSA) and Phytosterols Oletate(PSO). PSA (10,50mg/kg.d) and PSO(10, 50mg/kg.d) were given respectively to mice bearing transplanted tumor S_{180} by intraperitoneal injection for 9 days. The inhibiting rate of tumor growth, thymus index, spleen index, activity of catalase were detected. Results showed that both PSA group could inhibit the growth of S_{180} cell efficiently and increase the activity of catalase in erythrocyte of S_{180} cell in mice, exhibiting dosage-depended. Effect of inhibiting tumor of PSO was better than PSA.

Evaluated the effect of phytosterol oletate (PSO) on the immune function of S_{180}-bearing mice. Fifty mice bearing transplanted tumor S_{180} were used as animal model. The effects of PSO on the immune function were observed by intraperitioneal injecting different doses of PSO-1 (10mg/kg.d), PSO-2 (50mg/kg.d) and cyclophosphamide (20mg/kg.d) to mice respectively. Compared with the S_{180}-bearing mice model group, PSO could enhance significantly the phagocytosing ratio and the phagocytosing index ($\rho<0.05$), the effects of S_{180} were better than cyclophosphamide; PSO also could improve evidently delayed-type hypersensitivity ($\rho<0.05$), but worse than cyclophosphamide; PSO could increased formation of antibody in splenic cells of S_{180}-bearing mice, but there is not remarkable different ($\rho>0.05$). The results showed that PSO could improved immunologic function of mice.

7. Study on application of plytosterols ester

We have studied the soluble capacity of phytosterols ester and phytosterols. There are significant different in the soluble capacity among three phytosterols esters. Solubility(g/100mL, 20ºC) of phytosterols in soy salad oil was 1.55, PSO was 31.8, PSA was 8.07, PSS was 1.82; The soluble capacity of solubility was: PSO>PSA>PSS>Phytosterols.

The functional mayonnaise could be make up by adding PSO(2.5%) and natural tocopherol(0.5%) in the ordinary mayonnaise food. The optimum ingredients of the functional mayonnaise product were as follows: special soy salad oil(include PSO 3.5%, V_E 0.7%) 74 shares, yolk 14 shares and vinegar 9 shares.

References

A.E.Brynes, R. Mandeno. (2002). Acomparison of the LDL-lowering efficacy of plant sterol and stanol esters. Atherosclerosis Supplements. 3(2):176-178.

A.M.Wolfreys and P.A.(2002). Safety evaluation of phytosterol esters. Food and Chemistry Toxicology. 40(4): 461-470.

Award A B, et al.(2000) Dietary phytosterel inhibits the growth and metastasis of MDA-MB-231 human breast cancer cells grown in SCID mice. Anticancer.20:821-822

Arjan J.H.Louter, Christina G. Bauer-Plank and Guus S.M.J.E.(2002). Analysis of plant sterol esters as functional food ingredients. Lipid Technology. 14(4): 87-89.

Chonglun Xie, Laura A.,Woollett, Stephen D.(2002). Fatty acids differentially regulate hepatic cholesteryl ester formation and incorporation into lipoproteins in the liver of the mouse. Journal of Lipid Research. 43:1508-1519.

Dutta, Paresh C, Normen, Lena.(1998). Capillary column gas-liquid chromatographic separation of Δ5-unsaturated and saturated phytosterols. Journal of Chromatography A. 816:177-184.

J.C.kim, B.H. Kang.(2002). Subchronic toxicity of plant sterol esters administered by gavage to Sprague-Dawley rats. Food and Chemical Toxicology. 40:1569-1580.

Lena Normen, Paresh Dutta.(2000). Soy sterol esters and β-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. American J of Clinical Nutrition. 71: 908-913.

Malcolm R Law.(2000). Plant sterol and stanol margarines and health. 173:43-47.

Yuji shimada. Enzymatic Synthesis of Steryl Esters of Polyunsaturated Fatty Acid. J.Am.Oil Chem.Soc. 76:713-717

Technological performances of low linolenic/high oleic rapeseed oils for food and non-food application

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Abstract

Results on the low linolenic rapeseed oils frying stability were presented at the 11th International rapeseed congress in 2003 (2000 and 2001 studies).

Additional studies were undertaken in 2003 and 2004 on rapeseed oils displaying modified fatty acid (FA) composition : low linolenic (LL) content (between 1,1 and 3%), high oleic (HO) content (76,5 %), or both low linolenic (2,5 and 2,7%) and high oleic content (75%) (LL/HO).

Six pilot plant processed samples of refined rapeseed oils were evaluated for chemical properties and involved in tests for food and non-food applications.

Six room-odor tests were carried out on four experimental oil samples used as frying oil. Check was sunflower oil. Intensity and quality of the flavour were ranked by the panellists after the first, fourth and eighth frying. The 2003 study confirmed that the effect of a higher oleic content is not significant on room-odor performances. Studies of 2003 and 2005 confirmed that the low linolenic rapeseed oil at 2,5 % ranked slightly bellow the sunflower oil (paint and fishy flavours detected at the 4th and 8th frying) meanwhile low linolenic oil at 1,1 % is equivalent and even better than sunflower oil.

Regarding the chemical alteration of the oils during frying, polar component content showed that the LL/HO rapeseed oil seemed to perform better, suggesting an effect of the total unsaturation level on this property.

Chemical and physical checks were also carried out at the laboratory for lubricant uses in 2001, 2003 and 2004 studies. Better performances for hydrolytic and oxidation resistances (equivalent to a 80 % oleic sunflower oil) were obtained with the oils LL/HO oils.

Trials have finally been conducted to check the ability of such modified FA rapeseed oils as raw material for biofuel after a trans-esterification stage. LL/HO oils appeared to improve of 3 to 5 points the cetane index (EN ISO 5165) against the industrial control meanwhile other parameters have not clearly improved, leading to conclude that such modified oils are not necessary at the present time for the biodiesel market.

These works showed that the new LL/HO rapeseed cultivars could allow to extend rapeseed oil uses in both food (frying in substitution to hydrogenated fats) and non-food applications like lubricants.

Key words : rapeseed, room-odor, linolenic acid, oleic acid, frying, oxidation stability

Introduction

When used in deep fat frying, rapeseed oil gives off an odor in the kitchen which is perceived to be unpleasant (room-odor) by the homeowner, who is accustomed to peanut or sunflower oils. France, due to its own regulation, is the only country in the world to exclude oils containing more than 2% of linolenic acid for deep-fat frying. Some previous works from France and Canada have already shown that low-linolenic rapeseed oils (LL type) had a significantly better behaviour in frying than a normal rapeseed oil ; this behavior is very close to that of a sunflower oil.

Moreover, a high linolenic acid content in rapeseed oil restricts non-food applications as lubricants in warm conditions.

Several experimental cultivars with low content of linolenic acid and high content of oleic acid (HO type) are actually proposed by the plant breeding with the objective to develop food and non-food utilizations. Two studies (2000 and 2001) led to define the minimal limit acceptable for linolenic acid in order to obtain equivalent performances in room-odor between rapeseed oils and sunflower oils. The oil with a linolenic acid content of 1.1% is equivalent to sunflower after the first, the fourth and eighth frying. In contrary, panelists judged the oils with 2.2% and 3.5% of linolenic acid to have significantly higher intensities for fishy and paints odors. A high content in oleic acid did not appear to improve the flavor quality: the oil with 2.2% of linolenic acid and 64.3% of oleic acid was equivalent to the oil with 1.9% of linolenic acid and 78.8% of oleic acid.

Further studies were undertaken in 2003 and 2004 on six rapeseed oils displaying modified fatty acid composition : low linolenic (LL) content (between 1 and 3%), high oleic (HO) content (>75 %), or both low linolenic and high oleic contents in order to evaluate their ability to be used in both food (frying in substitution to hydrogenated fats) and non-food applications like lubricants and biofuels.

Materials and methods

Crop and Processing. Five rapeseed cultivars were selected for their low linolenic content (between 1 and 3 %) and/or for

their high oleic content (more than 75%) and cropped in 2003 and in 2004 by a private company and by a French public lab. The experimental seeds were crushed in the oil pilot plant Creol. Crude oils were refined by Iterg. One additional sample of refined LL/HO rapeseed oil (commercial LL/HO oil) was supplied by a seed company. The fatty acids (FA) modified rapeseed oils were compared to commercial oils: conventional sunflower and rapeseed oils.

Quality assessment. Analytical determinations were done on crude oils and refined oils ; analysis of frying oils were also done : peroxyde values according to NF T60-220, oleic acidity according to NF EN ISO 660, fatty acids analysis by gas chromatography according to NF EN ISO 5509 and NF EN ISO 5508, iron and copper by atomic absorption (NF EN ISO 8294), phosphorus by atomic absorption (IUPAC 2.423), tocopherols and tocotrienols by high performance liquid chromatography (IUPAC 2.432)(ISO 9936), polar compounds in frying oils (NF EN ISO 8420).

Frying performances.

LL and LL/HO experimental oils were evaluated for their performances in frying.

Room-odor tests. Room odor tests allow an evaluation and a comparison, in confined conditions, of flavours from two frying baths during cooking of potatoes :volume of frying bath : 2.5 litters, 360 g of French fries by bath, temperature of the bath : 180°C, eight frying called F1 to F8 for each oil.

Evaluation. Frying odors are evaluated by each member of the Iterg panel (13 people) after the first, the fourth and the eighth frying. The global quality of odor is determined by a score between 2 and 10. Odors are described through five characteristics with a scale going from weak to strong : fruity, burnt, acrid, painty and fishy.

Biolubricant performances.

The LL, LL/HO but also the HO rapeseed oils were also assessed as vegetable oil bases for lubricants.

Results for the 2001 oils are also given for a most comprehensive analisis.

Several chemical and physical tests were made by an accredited private lab to check the ability of the oils in relation to lubricant applications : cinematic viscosity (ASTM D445) at 40°c and 100°c, viscosity index, pour point (ASTM D 97), anti-wear property by the HFRR test (ISO 12156, ASTM D 147), oxidation stability at 99°C of steam turbine oils by rotating bomb (RBOT) according to ASTM D 2272, oxidation and hydrolytic stability according to Baader tests at 95°C (60 ml of oil, copper spiral, 72 hours, agitation 25 rotations per minute (rtm)), demulsibility according to ASTM D 1401 (54°C, 40 ml of oil, 40 ml water, 1500 rtm during 5 minutes).

Biofuels performances.

The LL, LL/HO, and HO rapeseed oils were finally checked at the lab scale for biofuel use.

The rapeseed oil methyl esters (RME) were synthesised at the lab scale (ITERG in 2003 and a private lab in 2004) by methanolysis path using an alkaline catalysis (transesterification reaction).

The experimental RME were fully controlled according to the European standard for automotive fuels- fatty acid methyl ester for diesel engines requirements and tests methods EN 14214. Quality of the esterification was verified as required by the standard: acid index and acidity (mg KOH/:g) NF ISO 660, peroxide index (NF T60-220), water content Karl Fisher (NF ISO 8534), methyl esters content (NF T 60-703), methanol content (NF T60-701), free, mono, di and tri glycerides (ITERG methods).

Special attention was done to the following characteristics included in the requirements list: iodine index (NF EN 14111), cold filter plugging point (CFPG, EN 116) which is a climatic dependent parameter, oxidation stability through the accelerated oxidation Rancimat test (110°c, 10g/l, 3g; NF EN 14112) and Cetane index (EN ISO 5165), which were expected to be improved with the FA modification.

Finally the cold performance of the RME was assessed by the pour point measurement (ASTM 5771).

Results and discussion

Quality of the oils

Fatty compositions. The fatty acid composition of the refined rapeseed and sunflower oils is given in the table 1.

Table 1 : Fatty acid composition (%) of the modified rapeseed oils

F.A.	Oils 2003						Oils 2004	
	LL 1	LL 2.5	LL 2.7 /HO 75.4	HO 76.4	LL2.3 /HO 72.6 commercial	Sunflower (control)	LL 2.4/HO 76.8	Sunflower (control)
16:0	3.6	4	4.3	3.9	3.6	6	3.8	6.2
18:0	1.5	1.5	1.7	1.5	1.6	3.9	2.2	4
18:1	63.6	64.1	75.4	76.4	72.6	29.4	76.8	25
18:2	26.7	23.3	12.7	7.6	15.7	58.8	11	62.2
18:3	1	2.5	2.7	6.8	2.3	0.1	2.4	0.1
20:0	0.6	0.6	0.6	0.5	0.6	0.3	0.8	0.3
20:1	1.4	1.7	1.3	1.5	1.5	0.1	1.3	0.2
22:1	<0.1	0.6	<0.1	0.2	0.6	<0.1	<0.1	0.1

Quality assessment of refined oils. The quality of experimental refined oils were in accordance with the rule of the Codex Alimentarius (tables 2 and 3). The peroxyde and oleic acidity values are largely below the Codex limits: 10 meqO2/kg for the peroxyde value and 0.3% for the oleic acidity. The refining has eliminated the prooxidant metals (iron and copper) the contents of which are also largely below the Codex limits. The degumming has eliminated phosphorus (content below detection limits, fixed at 5 ppm).

Table 2 : Quality parameters of the experimented rapeseed oils-

Oils Tests 2003	Peroxide value (meqO2/kg)	Oleic acidity (%)	Iron (mg/kg)	Copper (mg/kg)	Phosphorus (mg/kg)
LL 1	< 0.1	0.03 ± *0,01*	0,05	0,01	< 5
LL 2.5	< 0.1	0.01 ± *0,01*	0.071	0.01	< 5
LL 2.7/HO 75.4	< 0.1	0.04 ± *0,01*	0.012	0.008	< 5
HO 76.4	< 0.1	0.02 ± *0,01*	0.18 ± *0.09*	<0,005	<5
LL 2.3/HO 72.6 commercial	6.3± *2,5*	ND	ND	ND	ND
Sunflower (control)	3,6 ± *1,5*	0.02 ± *0,01*	<0.005	<0,005	< 5
Tests 2004					
LL 2.4/HO 76.8	< 0.1	0.03 ± *0,01*	0.048	0.006	< 5
Sunflower (control)			0.007	0.005	< 5

ND : non determined

Table 3 : Tocopherol content of the modified rapeseed oils (mg/kg)

oils	α		β	γ		δ	**Total**
Tests 2003	% of total			% of total			
LL 1	183	33.4 %	<DL	358	65.4 %	6	547 ± *82*
LL 2.5	160	31.3 %	<DL	344	67.3 %	8	511 ± *77*
LL 2.7 /HO 75.4	192	35.8 %	<DL	337	62.8 %	8	536 ± *80*
HO 76.4	200	34.8 %	< DL	368	64 %	8	575 ± *86*
LL2.3 /HO 72.6 commercial	242	35.6 %	< 2	428	62.9 %	11	680 ± *102*
Sunflower (control)	659	94.8 %	23	10	1.4 %	3	695 ± *148*
Tests 2004							
LL 2.4 /HO 76.8	234	40.2 %	3	334	57.5 %	10	581 ± *87*
Sunflower (control)	684	95 %	26	8	1.1 %	2	720± *108*

DL = detection limit

The total tocopherol content of the modified rapeseed oils is similar to the content of the conventional rapeseed refined oil (500 to 870 ppm, depending on the seed quality and on the refining) – (Table 3). The composition is not affected by the fatty acid profile modification, the γ type remaining predominant (57-65%) and the α type ranking second (30-40%); in accordance with the bibliography (α-tocopherols : 25-38%, β : 0-5 %, γ : 62-70%, δ : 0-6 %, source : Manuel des Corps Gras, Lavoisier TEC & DOC, p. 131, 1992).

Frying performances

Quality assessment of frying oils. The oleic acidity of refined oils and the content of polar components were determined after the first F1 and the eighth frying F8 (table 4). Few free fatty acids have been produced in rapeseed and sunflower oils (see the oleic acidity). After 8 frying, the polar components content is in conformity with the French regulation : less than 25% of polar components for food uses. After the first frying F1, the polar components content is in conformity with what is traditionally observed with rapeseed oils : 3 to 4% of polar components. Nevertheless after the 8th frying, the best result is got with the d ouble traits LL/HO rapeseed oil in both tests 2003 and 2004 suggesting a favourable effect of the linolenic acid content but also of the total unsaturation decreases.

Table 4 : Oleic acidity and polar components in frying oils after first (F1) and eight frying (F8) – tests 2003 and 2004

Oils	Oleic acidity (% m/m)		Polar components (%)	
	after F1	after F8	after F1	after F8
Test 2003				
LL 1	0.03 ± *0.01*	0.10 ±*0.01*	4.1 ± *1.5*	13.3 ± *1.5*
LL 2.5	0.01 ± *0.00*	0.05 ± *0.01*	3 ± *1.5*	10.9 ± *1.5*
LL 2.7/HO 75.4	0.005 ± *0.01*	0.08 ± *0.01*	3 ± *1.5*	6.8 ± *1.5*
Sunflower (control)	0.02 ± *0.01*	0.07 ± *0.01*	5,9 ± *1.5*	11.5 ± *1.5*
Test 2004				
LL 2.4/HO 76.8	0.05± *0.01*	0.08 ± *0.01*	3.2 ± *1.5*	7.6 ± *1.5*
Sunflower (control)	0.03± *0.01*	0.05 ± *0.01*	5.5 ± *1.5*	13.5 ± *1.5*

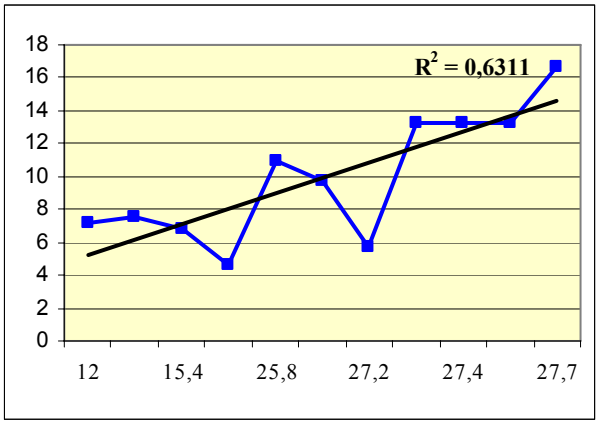

Figure 1 :Polar components (% m/m) in the modified rapeseed oils after the eight frying in relation to the C18:2+C18:3 content (%) – tests 2000, 2001,2003, 2004

NB : One value from the 2002 study (LL 2.2) was not taken into consideration

If we consider the results of all the tests run with the LL and LL/HO rapeseed oils from the year 2000, a relation ship between the polar components at F8 and the sum of 18:3 and C 18:3 contents can be established with a correlation coefficient of 0.63 (figure 1).

Room odor

The best ranking is obtained by the LL 1% oil; confirming the study of 2000 and 2001 where such fatty acid profile was shown as equivalent to sunflower (figure 2).

The addition of the high oleic trait (LL 2,7 % /HO 75,4 % in the 2003 tests and LL 2,4% /HO 76,8 % in the 2004 test) did not lead to improved flavour performances; confirming the major role played by the linolenic content on the frying oil flavour.

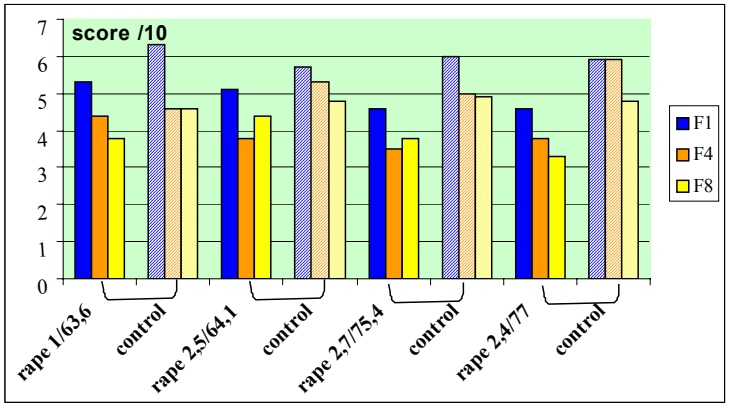

Figure 2 : Global quality assessment of the flavour after the first F1, fourth F4 and eighth frying F8 of the modified rapeseed oils tested in 2003 and 2004 in comparison to the conventional sunflower oil (control)

The results of the Student test for the four flavour descriptors and for the global quality assessment of the flavour at F1, F4 and F8 showed that the modified oils displaying 2,5% and 2,7 % of linolenic acid expressed some unfavourable flavours, especially painting fishy tastes for the LL 2,7 %/HO 75,4%, at the successive frying runs (table 5 and table 6).

These results confirm our previous study where the 2.5% C18:3 value was pointed as a kind of threshold over which some flavour differences can be detected in comparison to the sunflower oil.

Table 5 : Statistical results (Student test) of the room-odor tests 2003

Tested couple : Rapeseed oil against control (sunflower)	LL 1			LL 2.5			LL 2.7 /HO 75.4		
	F1	F4	F8	F1	F4	F8	F1	F4	F8
Global quality	1%	ns	ns	ns	1%	ns	1%	1%	ns
Lost of fruity	ns	ns	ns	ns	5%	ns	ns	1%	ns
Bean	ns	ns	ns	ns	ns	ns	ns	ns	ns
Burnt, acrid	ns	ns	ns	5%	ns	ns	ns	ns	ns
Painty, fishy	ns	ns	ns	ns	5%	ns	5%	1%	5%

ns : non significant

Table 6 : Statistical results (student test) of the room-odor test 2004

Tested couple : Rape oil against control (sunflower)	LL 2.7/HO 75.4		
Descriptor	F1	F4	F8
Global quality	5%	1%	1%
Lost of fruity	1%	5%	ns
Bean	ns	5%	ns
Burnt, acrid	ns	ns	ns
Painty, fishy	5%	1%	5%

ns : non significant

Nevertheless, it has to point that the differences were slight, that in this kind of design the score is highly related to the control oil which the rapeseed oil is compared to and that the people of Iterg involved in our room odor evaluation were hardly trained and sensitive. Additional consumers tests would be necessary to check the linolenic content which would be widely accepted for home frying use in France.

Lubricant performances

Physical and chemical characteristics.

The rapeseed oils experimented from 2001 till 2004 expressed a good viscosity index (in relation with a good lubricity) and a high flash point (reducing fire risks), in accordance with the fluid vegetable oils properties (table 7). The pour point was stable between years (-21 till -27°c) and was slightly better than the controls, however as for most vegetable oils, an additive should be necessary, depending on the conditions of use. (table 7, table 8).

The Noack volatility was very low and similar to the controls, in accordance with the behaviour of the fluid vegetable oils and their ability to decrease the lubricant dispersion in the atmosphere (good for the health impact on the operators) (table 7, table 8).

Table 7 : Physical and chemical properties of the rapeseed oils – tests 2001, 2003 and 2004

	Oils 2001			Oils 2003					Oil 2004
	LL 1.1	LL 1.9 HO 78.8	HO 77.1	LL 1	LL 2.5	LL 2.7/ HO 75.4	HO 76.4	LL 2.3 /HO 72.6 commercial	LL 2.4 HO 76.8
Viscosity at 40°c (mm^2/s)	35.91	38.64	36.65	35.78	33.38	37.49	36.73	37.38	37.89
Viscosity at 100°c (mm^2/s)	7.88	8.32	8.21	8.17	8.1	8.3	8.27	8.34	8.37
Viscosity index	200	199	209	214	230	210	211	209	206
Pour point (°c)	-27	-21	-21	ND	-23	-23	-22	-23	-20
Flash point (c°)	200	239	187	ND	ND	ND	ND	ND	ND
Noack volatility (%)	0.34	0.25	0.51	ND	ND	ND	ND	ND	ND

ND : non determined

Table 8 : Physical and chemical properties of the controls: commercial conventional rapeseed oil and sunflower oils (without additives)

	Conv rapeseed	**HO 80 sunflower**	**HO 90 sunflower**
Viscosity at 40°c (mm^2/s)	39.8	39.9	38.8
Viscosity at 100°c (mm^2/s)	8.9	8.7	9
Viscosity index	213	205	224
Pour point (°c)	-18	-12	-18
Flash point (°c)	269	265	272
Noack volatility (%)	0.4	0.37	0.3

Lubricants tests

The first tests of 2001 had highlighted a good anti-wear property of all the rapeseed oils whatever the LL and HO modification, in accordance with the literature references available for fluid vegetable oils. That's why we did not replicate such tests in the following years (Table 9).

The modified rapeseed oils expressed also acceptable water separation properties (table 9) with average times of 15-20 minutes before obtaining the separation of the oil base from the water.

Regarding both oxidation tests (RBOT at 40°c and Baader test), the LL modification did not lead to a clear improvement of the performances compared to the commercial conventional rapeseed oil. (Table 9, figures 3,4)

The best results, equivalent to the 80% oleic sunflower oil, were obtained by experimental oils combining LL and HO traits, suggesting a favourable effect of the total unsaturation decrease and of the linolenic acid lowering as for the oil frying oil stability (figures 3,4).

However none of the evaluated rapeseed oils reached the performances of the 90% oleic sunflower oil, suggesting that

the hydrolytic and oxidation stabilities of such modified rapeseed oils remain insufficient for the most sensitive lubricant uses (some hydrolytic and all the motor oils lubricants)- figures 3 and 4

Table 9: Physical and chemical properties of the modified rapeseed oils

	Oils 2001			Oils 2003					Oil 2004
	LL 1.1	LL 1.9/ HO 78.8	HO 77,1	LL 1	LL 2.5	LL 2.7/ HO 75.4	HO 76.4	LL 2.3/ HO 72.6 com.	LL 2.4/ HO 76.8
Wear test HFRR (mm)	0.68	0,.	0.69	ND	ND	ND	ND	ND	ND
Demulsibility ml oil/ml water/ml emulsion/(min)	43/36/1 (20)	42/37/0 (15)	43/37/0 (15)	40/40/0/15	40/40/0 /15	40/40/0/ 15	41/37/2/ 15	40/38/2/ 25	41/37/2/20
Oxidation stability RBOT at 99°C(min)	64	125	90	91	97	99	100	97	130
Oxidation stability Baader, variation of TAN (mg KOH/g)	0.15	0.24	0.38	0.001	0.475	0.34	0.60	0.5	0.26
Oxidation stability Baader, variation of viscosity at 40°C	29.29	20.52	28.05	39.55	49.7	25.85	22.6	36.44	25.73

ND : non determined

Table 10 : Physical and chemical properties of the controls.

	Conv rapeseed	HO 80 sunflower	HO 90 sunflower
Demulsibility ml oil/ml water/ml emulsion/(min)	30/40/1 (20)	40/40/0 (20)	40/40/0 (20)
Oxidation stability RBOT at 99°C (min)	61	130	>250
Oxidation stability Baader, variation of TAN (mg KOH/g)	0.43	0,3	0
Oxidation stability Baader, variation of viscosity at 40°C	29.5	20	14.2

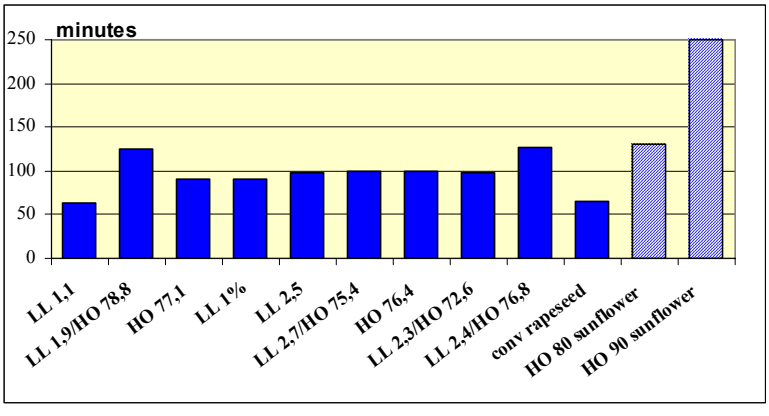

Figure 3 : Oxidation stability, RBOT test at 99°c, time to obtain a pressure depression of 175 KPa, tests 2001, 2003 and 2004.

Biofuels performances

All the methyl esters obtained from the modified rapeseed oils fitted a good quality.

The iodine index of the LL or HO RME is improved compared to the control and is fare bellow the maximum limit of 120 included in the FAME biofuel standard CEN 14214.

The LL or HO RME delivered good cold performances (pour point and CFPP) similar to the controls.

The cetane index of the FA modified rapeseed oils was found to also meet the European standard CEN 14214 (>51) and to be improved with the decrease of LL content and/or the increase of the HO content. In the 2003 tests, the double modified type (LL/HO) surpassed the other modified rapeseed RME for the cetane index.

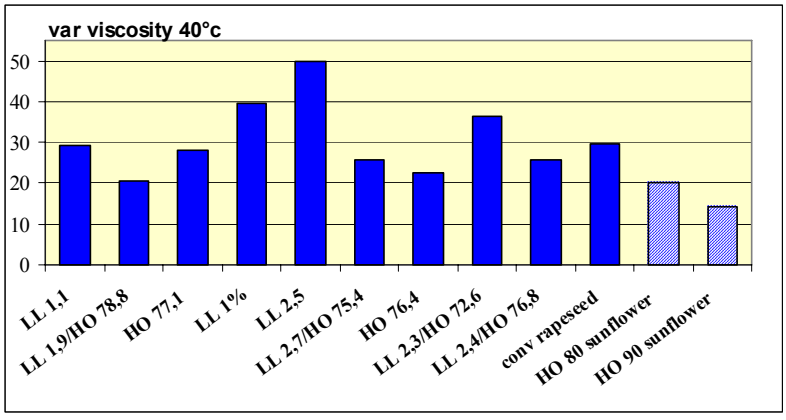

Figure 4 : Oxidation stability in the Baader test at 95°c; variation of the viscosity measured at 40°C before and after the test in the 2002, 2003 and 2004 experiments.

First reliable results in the Rancimat test were obtained in 2004 suggesting a better oxidation stability of the LL/HO rapeseed oil type compared to the conventional one. Further controls would be necessary to quantify the difference.

All in all the LL/HO modification of the rapeseed oil could be a way of biofuel quality improvement.

Table 11 : Physical and chemical characteristics of the modified rapeseed methyl esters (RME)

	RME 2003					RME 2004	
oils	LL2.5	LL2.7/ HO 75.4	HO 76.4	LL 2.3/ HO 72.6 commercial	Control Commercial RME	LL 2.4/ HO 76.8	Control commercial RME
Iodine value	111	99	94	ND	ND	96 ± *3*	117 ± *3*
Peroxide index (meq O2/kg)	9	3.3	3.95	1.5 ± *2*	5 ± *2*	2.5± *1*	2± *1*
CFPP (1) (°c)	-17	-15	-19	- 16	- 10	-10	- 13
Pour point (°c)	- 12	- 10	- 13	- 21	- 15	-11	- 12
Trouble point (°c)	- 4	- 4	- 5	- 2	- 3	- 2	- 7
Cetane index	58.3	59	56.75	53.3	50.1	53.9	51.8

(1) : cold filter plugging point
ND : non determined

Table 12 Oxidation stability of the modified rapeseed oil evaluated in 2004

	RME 2004	
	LL 2.4/HO 76.8	Control commercial RME
Oxidation stability (Rancimat), induction time in hours	18.5 ± *2.8*	8.1 ± *1.5*

NB : Commercial RME is not additivated

Conclusion

The new low linolenic and high oleic rapeseed cultivars provided in Europe by the breeding activity confirmed improved performances of the oil in hot conditions compared to the conventional FA profile. Regarding frying behaviour, the quality flavour was confirmed to be acceptable until 2 to 3 % of linolenic acid in the oil in our experiments and was not dependent on the oleic content. These results would have to be confirmed in consumer tests.

The association of high oleic (> 75%) and low linolenic (< 2.7 %) traits gave the best technological performances of the oil, especially for oxidation stability in both food and non-food evaluation. Therefore we can expect for the LL/HO rapeseed oils new markets in the frying food as well as in the lubricant fields. Such cultivars will also offer a way to enhance the quality of the biofuel while being processing as methyl esters.

To conclude may we imagine that in a near future the low linolenic/high oleic rapeseed varieties will become the commodity type meanwhile the conventional profile (with 7-10 % of linolenic acid) will move to a speciality type for their nutritional value ?

Acknowledgments

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References

P. Carré, C. Dartenuc, J. Evrad, A. Judde, F. Labalette, M. Renard, R. Raoux : Frying stability of rapeseed oils with modified fatty acid compositions. In : proceedings of the 11th *International Rapeseed Congress*, 2003, 540-543

J. Kristott : High-oleic oils: How good are they for frying ? *Lipid technology*, 2003 March 2003 29-32

B. Matthäus : Utilzation of high oleic rapeseed oil for deep-fat frying of French fries compared to other commonly used edible oils. : *Eur. J. Lipid Sci. Technol.* 1008 (2006) 200-211

L. Normand, M. Eskin, R. Przybylski: Effect of tocopherols on the frying stability of regular and modified canola oils. *J Am Oil Chem Soc.* 2001, 78 n°4, 369-373

R. Przybylski, M. Eskin, L. Normand : Frying performance of modified canola oils. In *10th International Rapeseed Congress*, 1999, 26-29 sept, Canberra, Australia

I. Petukhov, LJ Malcolmson, R. Przybylski, L. Armstrong: Frying performance of genetically modified canola oils *J Am Oil Chem Soc* 76, n°5, 627-632

X. Q Xu, V. H. Tran, M palmer, K White, Ph. Sallisury: Chemical and physical analyses and sensory evaluation of six deep-frying oils. *J Am Oil Chem Soc*. 1999, 78, 1091-1099

C. Su, P. White : Frying stability of high oleate and regular soybean oil blends. *J Am Oil Chem Soc.* 2002, 81 N°8- 783-788

K. Warner, P. Orr, M Glynn: effect of fatty acid composition of oils on flavour and stability of fried foods. *J Am Oil Chem Soc*. 1997, 74 347-356

Rapeseed/canola protein isolates for use in the food industry

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Abstract

Burcon has developed and patented a process that is significantly different from conventional protein purification technologies that deal with plant protein sources. The Burcon process uses only water and salt to extract the proteins from the meal and it does not require the conventional isoelectric precipitation. Burcon's process is based around a micelle formation step, which results from a reduction in the ionic strength of the protein solution. Rapeseed/canola protein isolates produced by this original process, which does not require any harsh chemicals, are superior in their organoleptic as well as their physical functional properties compared to canola proteins made through traditional methods. Currently, there are two protein products available: Puratein© and Supertein™.

Key words: Rapeseed protein isolate, extraction, protein functionality, Puratein®, Supertein™

Introduction

Rapeseed/canola is the second largest oilseed crop in the world and is an excellent source of protein with its meal having protein concentrations up to 40%. The meal, which is left after oil extraction, is currently almost exclusively used as animal feed, although, with a balanced amino acid composition and appreciable levels of sulphur amino acids, rapeseed protein would be an excellent human food source. According to a recent study (Millward, 2006), rapeseed protein garners a protein score of 100% based on the new FAO/WHO age-related scoring patterns for children and adults. The nutritional value of rapeseed proteins has also been found to compare favourably with that of animal proteins (Friedman, 1996).

Although many studies have been published concerning rapeseed/canola protein, attempts to make an acceptable rapeseed/canola protein product commercially available for the food industry have been unsuccessful. Protein isolates made through conventional technology usually have undesirable flavour and colour and are limited in their physical functional properties. Typically, researchers have described an alkaline extraction followed by an isoelectric precipitation step (Rutkowski, 1975; Chen et al., 1992; Vioque et al., 1999). However, the major drawback of alkaline extraction is that oxidation of phenolics, which are extracted from the meal along with the protein, results in the unacceptable colour and flavour of the final protein product. There are also other extraction solvents described in the literature, such as a methanol/acetone/water mixture (Lacroix et al., 1988), a solution of sodium hexametaphosphate (Tzeng et al., 1988) or a solution of copper sulphate (Kroll et al., 1991).

This paper describes a new patented process for the production of rapeseed/canola protein isolates that overcomes the hurdles of traditional processes.

Materials and Methods

Rapeseed protein isolate production

Rapeseed protein isolates were produced using a proprietary process. Rapeseed meal was prepared using a pilot-scale oilseed crushing and extraction facility. A salt solution was used to extract rapeseed protein from the rapeseed meal. The protein depleted meal was removed by several purification steps producing a clarified extract. This extract was subjected to an ultrafiltration step, through which the protein solution was concentrated and purified.

The concentrated protein solution was then diluted into cold water, which caused a fraction of the proteins to aggregate in the form of micelles. As the micelles aggregated, they settled out and were removed by decantation or centrifugation. The protein micellar mass that was removed from the dilution step was spray dried to form the first canola protein isolate product (Puratein®). The supernatant from the dilution was further processed and finally spray dried to generate the second rapeseed protein isolate product (Supertein™). Figure 1 shows a general scheme of the protein production process.

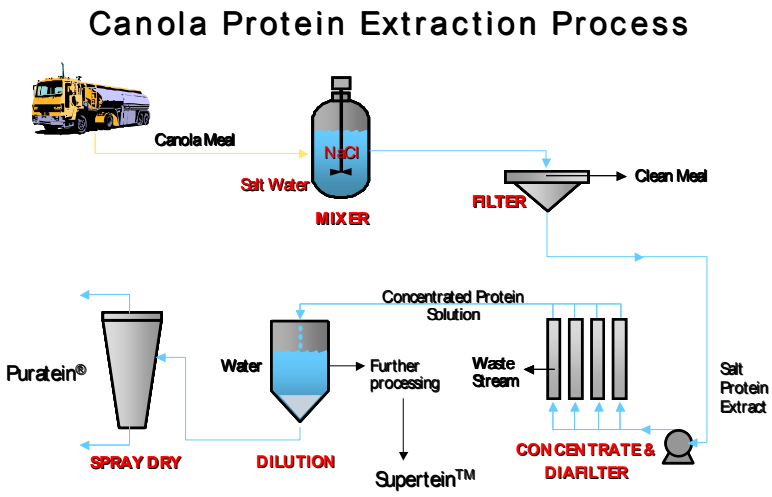

Figure 1. Scheme of Burcon's Canola Protein Isolate Production Process

Functionality testing

Functional properties were evaluated by basic testing and also demonstrated by preparing model food systems. Solubility of Supertein™ was tested in water at different pH values and also demonstrated in various acidic beverages including soft drinks and sports drinks. The ability of Supertein™ and Puratein® to produce foams was evaluated by whipping 5% w/v protein solutions for various lengths of time. The foam properties of Supertein™ were demonstrated by preparing nougat, while the heat stability of Puratein® foam was demonstrated by preparing foam and chocolate cakes. Gelling of Puratein® was evaluated by puncture testing gels prepared by heating 8% w/w dispersions of Puratein® in water for 90°C for 30 minutes. Ingredient binding capabilities of Puratein® were demonstrated by preparing chocolate chip cookies and vegetable (bean) burgers.

Results and discussion

Protein Isolate Production

The proprietary protein extraction and purification process resulted in two rapeseed protein isolates having protein concentrations greater than 90% on a dry basis. The high purity of the protein products was largely attributable to the ultrafiltration step as well as the dilution step. By using suitable membranes for the ultrafiltration step, the non-protein components were separated from the proteins. Ultrafiltration and similar selective membrane techniques permit low molar weight species to pass through into the permeate while retaining higher molar weight species in the retentate. The low molar weight species removed in the production of the canola protein isolates included not only the ionic species of the food grade salt but also low molar weight materials extracted from the meal, such as, carbohydrates, pigments etc.

The elimination of low molar weight species from the extracted solution without a substantial change in the ionic strength permitted the protein concentration to be increased without precipitation. Diluting the concentrated protein solution into cold water caused a reduction of ionic strength that resulted in the aggregation (Figure 2) of the high molar weight proteins while the low molar weight proteins stayed in solution. The proteins precipitated in the form of micelles as can be seen in Figure 3.

Figure 2. Precipitation of rapeseed proteins

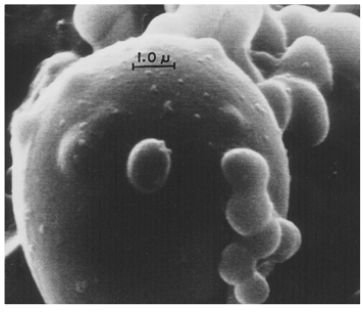

Figure 3. Electronic microscope picture of rapeseed protein micelles.

As is well reported, rapeseed contains two major proteins fractions: globulins, represented by cruciferin, and albumins, represented by napin. It appears that Burcon's process separated these two fractions very well and the majority of the globulin fraction was found in the Puratein® while the majority of the albumin fraction was found in the Supertein™. Table 1 shows the

composition of the two protein isolates.

Table 1. Composition of Rapeseed Protein Isolates

	Supertein™	Puratein®
Protein (N*6.25)	> 90% d.b.	> 95 % d.b.
Protein fraction	Majority: Albumins	Majority: Globulins

Functionality of Supertein™

The amino acid composition of Supertein™ was found to be especially rich in sulfur containing amino acids, which is particularly valuable from a nutritional standpoint.

Supertein™ was highly soluble and produced transparent solutions, even in acidic pH conditions. This makes it an ideal protein for use in fortified beverages. When compared with other available proteins such as soy, whey and egg white, Supertein™ was the only protein that formed a transparent solution in an acidic soft drink. Additionally, Supertein™ was very heat stable, which facilitates pasteurization where needed.

Supertein™ was found to form large volumes of foam with good foam stability. The foaming properties of Supertein™ compare favourably with those of egg white proteins. Acceptable nougat was produced with Supertein™

The flavour of Supertein™ has been observed to be bland and free of off-notes.

Functionality of Puratein®

Puratein® has been found to be a good emulsifier. A spoonable dressing with 67% oil was prepared with only 0.11% Puratein® as the emulsifying agent.

Puratein® was found to form strong heat induced gels at low acid conditions, performing better as a gelling agent than soy protein isolate.

Chocolate cake and foam cake were produced with Puratein® instead of egg ingredients, resulting in cakes with acceptable volume and texture. This demonstrated the ability of Puratein® to retain air when baked.

Puratein® was used to replace whole egg in chocolate chip cookies and vegetable burgers, showing its ability to function as an ingredient binder.

Puratein® was observed to have a very bland flavour with no off flavours noted.

Conclusion

Burcon's protein production The process is very clean and gentle, using only water, salt and rapeseed/canola meal. The process results in two rapeseed/canola protein products, Supertein™ and Puratein®, which are commercially viable products with a variety of functional properties. Applications for Supertein would include but are not limited to fortified beverages, nutrition bars and aerated desserts and confections, while applications for Puratein would include but are not limited to dressings, sauces, meat substitutes and baked goods. The findings suggest that rapeseed/canola proteins have the potential to open up some new food applications and to compete with major animal or plant proteins.

References

Chen M. and Rohani S. (1992). Recovery of Canola Meal Proteins by Precipitation, Biotechnology and Bioengineering. **40**, 63-68

Friedman, M. (1996). Nutritional value of proteins from different food sources. A review. J. Agric. Food Chem., **44**, 6-29

Kroll J., Kujawa M. and Schnaak W. (1991). Preparation of rapeseed proteins by extraction and ultrafiltration. Fat Sci. Technol., **93 (2)**, 61-65

Lacroix M., Amiot J. and Cheour F. (1988). Effect of methanol/acetone/water extraction and enzymatic hydrolysis on the nutritional value of unheated rapeseed proteins. Plant Foods for Human Nutrition. **38**, 343-353

Millward D.J. (2006). Oilseed Rape Protein in Human Nutrition. Proceedings of the Workshop Oilseed Rape for a healthier future, 30th September – 1st October, Madrid, Spain

Rutkowski A. (1975). "Technologische Richtlinien bei der Verarbeitung von Rapsproteinen zu Eiweiss konzentraten. Die Nahrung, **8/9**, 941-954

Tzeng Y.M., Diosady L.L. and Rubin L.J. (1988). Preparation of Rapeseed Protein Isolate by Sodium Hexametaphosphate Extraction, Ultrafiltration and Ion-Exchange. J. Food Sci, **53 (5)**, 1537-1541

Vioque J., Sanchez-Vioque R., Clemente A., Pedroche J, Bautista J. and Millan F. (1999). Production and Characterization of an Extensive Rapeseed Protein Isolate. JAOCS, **76 (7)**, 819-823

Chlorophyll reduction in rape seeds and it's influence on the bleaching earth consumption during refining

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Abstract

It is well known, that chlorophyll in edible oils reduces their oxidation stability, taste and shelf-life. In rape seeds chlorophyll is newly synthesised about 3 – 6 weeks after pollination. Short vegetation times, dryness, coldness or early winter onset influence the chlorophyll metabolism negatively.

The effect of the chlorophyll content in rape seeds on the bleaching earth consumption during the refining process was measured under lab scale conditions. It could be shown, that even a low decrease of the crude oil chlorophyll content in harvested rape seeds can lead to considerable bleaching earth savings.

Therefore different strategies to reduce chlorophyll in rape seeds were investigated.

These are classical plant breeding methods as well as the development of transgenic plants by inactivation of chlorophyll producing enzymes or over expression of chlorophyll degrading enzymes.

Finally it was tried to remove chlorophyll from crude rape seed oil by enzymatic treatment using recombinant proteins under refining conditions.

Key words: Rapeseed, oil, chlorophyll, refining, bleaching earth, plant breeding, transgenic plant, enzymatic process

Introduction

Chlorophyll is the green pigment in chloroplasts of plants and essential to photosynthesis. Therefore, there are small quantities of chlorophyll found in nearly every type of vegetable oil. But, the level of chlorophyll in rapeseed oil is mostly higher than that found in other vegetable oils. Especially in the case of freezing before the rapeseed has significantly ripened, the chlorophyll content in the crude rapeseed oil is very high and can be a problem for the refineries. We know this from Canada, North and East Europe. The reason is, that chlorophyll is synthesised again within the rape seed about 3 to 6 weeks after pollination.

Chlorophyll in edible oils reduces their oxidation stability, taste and shelf-life. To achieve stable oils, it is necessary to decrease the chlorophyll content in the oil to 0.05 ppm. Although up to 30 % of the total amount can be removed by neutralisation in the case of chemical refining, bleaching is the most important chlorophyll reducing refining step.

Worldwide about 45 mill. t/a rapeseed, or about 16 mill. t/a rapeseed oil, are produced. If it is assumed, that 0.7 % bleaching earth is needed for rapeseed oil bleaching, about 110.000 t/a of bleaching earth worldwide is applied for rapeseed oil refining.

The first aim of the studies was to determine to which amount bleaching earth can be saved only by reducing the chlorophyll content in the crude rapeseed oil (resp. the rape seeds).

At second, different strategies to reduce the chlorophyll content in rape seeds were investigated:

- classical plant breeding,
- development of transgenic plants, by
  a) inactivation of chlorophyll producing enzymes or
  b) overexpression of chlorophyll degrading enzymes.

Finally, the use of recombinant proteins to develop an enzymatic chlorophyll removal process from the oil was studied. The main questions of the investigations were:

- Are the enzymes active in oil?
- Which are the optimal reaction conditions?
- Where (regarding to the refining process) the enzyme is to put into the oil?
- Is the enzyme able to harm the oil?

Materials and Methods

1. Refining experiments in laboratory scale were carried out. Because, there were no crude rapeseed oils with high chlorophyll content available, model oils were made. For that, to the oil either pure chlorophyll or refined rapeseed oil was added to increase or decrease the initial chlorophyll content of the oil to a range between about 2 and 20 ppm. These oils were bleached under different conditions. Bleaching earth consumption, bleaching temperature and time as well as the citric acid concentration were varied. After that, the oil was normally deodorised and later analysed.

2. Chlorophyll kinetics in the seeds of 39 different winter and 33 summer rape accessions were measured.

3. There are two enzymes involved in the synthesis of chlorophyll a: glutamat-1-semialdehyd-aminotransferase (GSA-AT) and magnesium-chelatase. The genes for these enzymes were amplified and isolated by PCR. Gene constructs for reduced gene expression by RNA interference were generated and used for rape hypocotyl transformation by *Agrobakterium tumefaciens*.

There are four different enzymes involved in the chlorophyll catabolism: chlorophyllase, Mg-dechelatase, pheophorbide-a-oxygenase and RCC reductase. First, only the chlorophyllase genes were amplified and cloned from *Citrus clemente*. Gene constructs were generated and used for rape transformation in the same way described before.

Totally, thirty nine transgenic rape lines were generated.

4. The use of recombinant proteins for an enzymatic chlorophyll removal from the oil was examined.

At first, the chlorophyllase gene was amplified and cloned from clementine. The protein was then expressed in *E. coli.* The soluble, insoluble and total protein fractions were assayed for enzymatic activity tests, carried out at different conditions of pH, temperature, substrate concentration and enzyme concentration. The effects were measured by thin layer chromatography.

After that, it was tried to transform these results into a process for enzymatic chlorophyll removal under laboratory conditions. Basis was the classical chemical edible oil refining process. Because there was no rapeseed oil rich in chlorophyll available, pure chlorophyll a were added to the crude oil. After degumming and neutralisation a buffer with pH 7.8 and a liquid chlorophyllase extract were added. The system was stirred at fifty five degrees for 2 hours. Then it was tried to separate the formed chlorophyllide into the water phase.

In addition, the gene for phaeophorbide-a-oxygenase was amplified and cloned from tomato and the gene for RCC-reductase was delivered from the University of Bern. Both were expressed in *E. coli*. Enzyme activities of soluble and unsoluble assays were proved.

Results

1. The crude oil chlorophyll content very strong influences the bleaching earth amount, which is needed to reach a target chlorophyll content in the refined oil. At higher crude oil chlorophyll contents significantly more bleaching earth is needed as at lower crude oil chlorophyll contents to reach the same chlorophyll content in the refined oil.

2. Winter rape lines contain generally lower chlorophyll than summer rape lines. Different types of kinetics indicate a potential for further breeding activities to develop rape lines with lower chlorophyll content in the seeds.

3. The GSA-AT RNAi gene constructs showed no effect. Two plants with Mg-chalatase-RNAi constructs were extremely chlorotic. Chlorophyll was not only removed from the seeds, but also from the leaves. All other plants showed no effect. In 4 plants with chlorophyllase overexpression gene constructs reduced chlorophyll contents were measured at the 42 day after pollination. In summary, the strategy of chlorophyll overexpression seems to be more promising, than the strategy of inhibition of the chlorophyll synthesis.

4. Enzymatic activity tests showed, that chlorophyllase from *Citrus* can be used in oily buffers, has a high thermostability, which is higher in oil than in water, and has low activity losses at pH values different from the optimum.

Lab scale enzymatic refining, using chlorophyllase, showed that the chlorophyllide can be formed, but not effectively separated. Within the activity tests acetone was used as a solubiliser. Because the use of acetone in edible oil refining is not possible, it was looked for other substances. Using a mixture of 1 part water and 1 part isopropanol, a slight separation was possible. Other systems, using ethanol or glycerine, failed.

Activity tests showed, that the used phaeophorbide-a-oxygenase and RCC-reductase are active, but the fluorenscent chlorophyll catabolite is only formed in the case of presence of both enzymes. Because both enzymes need some co-factors for working, a successful application into the edible oil refining process is not much probable.

Conclusions

Even low reductions of the chlorophyll content in crude rapeseed oil lead to a considerable decrease in refining expense, especially to a lower bleaching earth consumption.

Different chlorophyll reduction strategies were investigated.

Recombinant chlorophyllase from *Citrus clementii* is able to split the chlorophyll into chlorophyllide and phytol under normal edible oil refining conditions. For the separation of the hydrophilic chlorophyllide, an acetonic system is necessary.

The activity of recombinant pheaophorbide-a-oxygenase and RCC-reductase is proved in vitro. Because of the need of some co-factors, their application in an edible oil refining process is not to expect.

There were 39 transgenic plants generated. 4 of them showed significantly lower chlorophyll contents at the 42 day after pollination.

The chlorophyll kinetics of 35 summer and 39 winter rapeseed lines during ripening were measured. Large differences between them indicate a potential for classical plant breeding of rapeseed lines with low chlorophyll content.

High oleic low linolenic rapeseed oil as alternative to common used frying oils

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Abstract

Changes of high-oleic low linolenic rapeseed oil during 72 h of deep-fat frying of potatoes were compared concerning chemical, physical and sensory parameters with commonly used frying oils, palmolein, high-oleic sunflower oil and partially hydrogenated rapeseed oil. From a sensory point of view French fries obtained with HOLL rapeseed oil, palmolein and high-oleic sunflower oil, respectively, were still suitable for human consumption after 66 hours of deep-fat frying, while French fries fried in partially hydrogenated rapeseed oil were inedible after 30 h. During the frying period none of the oils exceeded the limit for the amount of polar compounds, oligomer triglycerides and free fatty acids, respectively, recommended by the German Society of Fat Science (DGF) as criteria for rejection of used frying oils. From the results it can be concluded that the use of high-oleic low linolenic rapeseed oil for deep-fat frying is comparable to other common used oils.

Key words: frying, high-oleic low linolenic rapeseed oil, high-oleic sunflower oil, palm olein, partially hydrogenated rapeseed oil

Introduction

Deep-fat frying is one of the most popular methods for the preparation of food. Nevertheless food being fried is in iscussion, because it contains a lot of oil from the frying medium. Since the oil becomes part of the food the nutritional value of the food strongly depends on the composition of the oil used for frying. Palm olein but also partially hydrogenated oils like hydrogenated rapeseed oil or peanut oil are common used for industrial but also homemade frying. From a technical point of view these oils are favourable, because of the low content of polyunsaturated fatty acids, which are critical concerning the oxidative stability of the oils. But a strong disadvantage of these types of oil is the high amount of unhealthy fatty acids such as saturated or *trans*-fatty acids which are taken up with products being fried. Palm olein is distinguished by a high amount of saturated fatty acids and in hydrogenated fats and oils high amounts of *trans*-fatty acids can be found, which are hold responsible for some negative effects on blood cholesterol resulting in coronary heart diseases (Precht and Molkentin, 1995; Stender et al., 1995). From this the perfect frying oil should be low in saturated and *trans*-fatty acids, high in mono-unsaturated oleic acid, stable against oxidative deterioration during use and the use of the oil should ensure the production of high-quality and tasty foods.

The paper describes the results of a frying experiment using high-oleic low linolenic rapeseed oil as frying medium in comparison to palm olein, high-oleic sunflower oil and hydrogenated rapeseed oil.

Materials and Methods

For the investigation fryers, usually applied in household were used. A frying temperature of 175°C was chosen. For the investigation 2.0 l of the appropriate edible oil were filled into the fryer and afterwards the oil was heated up to 175°C within 10 min. The oil was hold on this temperature for 1 h before 50 g pre-fried potatoes were fried 3.5 min.. Five frying operations were carried out each day with 50 g pre-fried potatoes, each. Between each frying step the fryer was hold at 175 °C for 1 h without frying material, resulting in a thermal load of the oil of 6 h per day.

At the end of each day the oil was cooled down, filtrated and 200 mL of oil were taken for the characterisation. The oil was stored at 6°C until further use. Next day 200 mL fresh oil was added and the experiment was repeated on eleven successive days. This resulted in a total thermal load of the oils of 72 hours.

The oils as well as the fresh French fries were characterized with regard to the sensory quality and also some chemical parameters, such as content of free fatty acids, polar compounds and oligomer triglycerides were used for the evaluation.

Partially hydrogenated rapeseed oil (PHRO), Palm olein (PA), high-oleic sunflower oil (HOSO) and high-oleic low linolenic rapeseed oil (HOLL) (NATREON™) were used as frying medium.

Table 1. Methods used for the evaluation of fresh and used frying oils

No.	Parameter	Method	Reference
1	Polar compounds	DGF C-III 3b	DGF, 2005
2	Oligomere triglycerides	Cd 22-91	AOCS, 1990
3	Free fatty acids	DGF C-V 2	DGF, 2005
4	Sensory assessment	modified DGF C-II 1 (97)	DGF, 2005

Results

An important parameter for the assessment of the frying process is the quality of the product being fried, because the sensory quality is responsible for the success of the product on the market. If the sensory sensation does not meet the expectation of the consumer it will be rejected. The sensory quality is not only the taste of the French fries but also the colour, the crust and the inner composition of the fried potatoes.

With continuing frying time the taste of the fried potatoes was judged poorer and poorer (Fig. 1). The French fries took on rancid aroma components and the taste turned into bitter, burnt and rancid. Especially French fries deep-fried with PHRO showed a strange smell and taste, which led to a clear devaluation of the products. This is not surprising, because it is known that hydrogenated frying oils impart a different type of flavour to food. French fries obtained with HOLL, PO and HOSO, respectively, were still suitable for human consumption after 66 hours of deep-fat frying, the taste was still satisfactory (assessment better than 6 (dotted line)). Only afterwards French fries were judged worse. At the beginning of frying, within the first 42 h the assessment of French fries fried in PO and HOSO, respectively, were evaluated better than French fries fried in HOLL. Then the results were comparable.

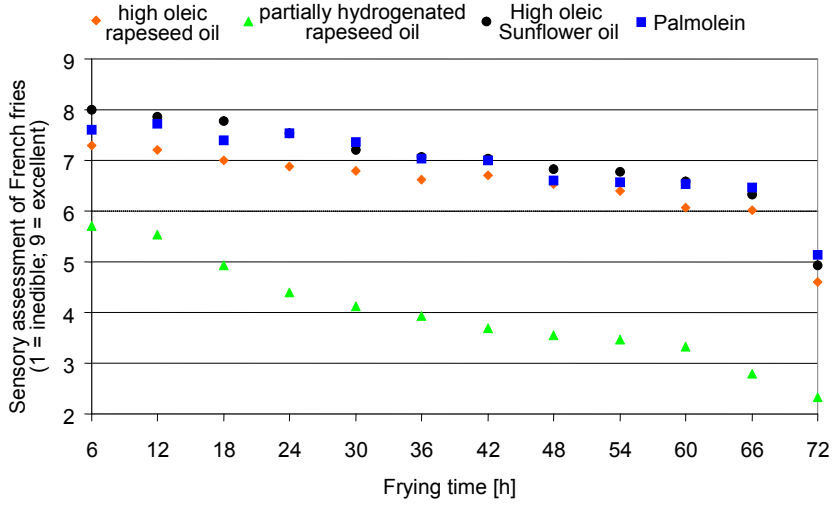

Fig. 1. Development of the sensory evaluation during a frying period of 72 h.

Looking on the other parameters for the characterisation of the fried products, like crust, inner composition or colour, all the oils showed acceptable results with regard to the colour of the French fries. The inner composition and the crust were in an adequate up to a good level over a frying period of 54 hours. For HOLL and PO, respectively, these parameters were even reasonable over a period of 66 h. Only the inner composition and the crust of PHOR were evaluated worse after only 30 h of frying (results not shown).

For the further assessment of the oils regarding their suitability as frying medium the recommendations of the German Society of Fat Science (Anonymous, 2000) were used: oligomer triglycerides, polar compounds (Fig. 2) and free fatty acids. Comparable to the polar compounds also for oligomer triglycerides and free fatty acids the amount increased linear with the frying time. But not any of the oils exceeded the limits given in the recommendation for these parameters during the frying period of 72 h, which would lead to the rejection of the oils. While the amount of polar compounds in HOLL, HOSO and PHRO was comparable, but significantly different ($p < 0.01$), in PO higher amounts of polar compounds were found, as a result of the higher initial content of this oil.

A summarized comparison of the used oils was carried out by distribution of rank numbers according to the come out of each oil at the most important parameters for the assessment of the oils after a frying time of 72 h. The oil coming out on the top got an 1 and the worst oil a 4. After distribution of the rank numbers the mean value was calculated for each oil. Additionally to the amount of polar compounds, oligomer triglycerides and free fatty acids as well as the sensory evaluation of the oil, the sensory evaluation of the potatoes being fried was used for the assessment, because this is one of the most important criteria for the consumer. The summary of this assessment is shown in Table 2.

No significant differences ($p < 0.01$) were found between HOLL, HOSO and PHRO concerning the total assessment of the results, even if the table shows that HOLL has clear advantages with regard to the sensory evaluation of both, oil and product. In contrast, PHRO had better results for the chemical parameters, but a bad sensory evaluation. Further on it should be pointed out that only small differences in the chemical parameters of all oils were found. Only the total result of the assessment of PO was significantly worst than the results of the other oils ($p < 0.01$).

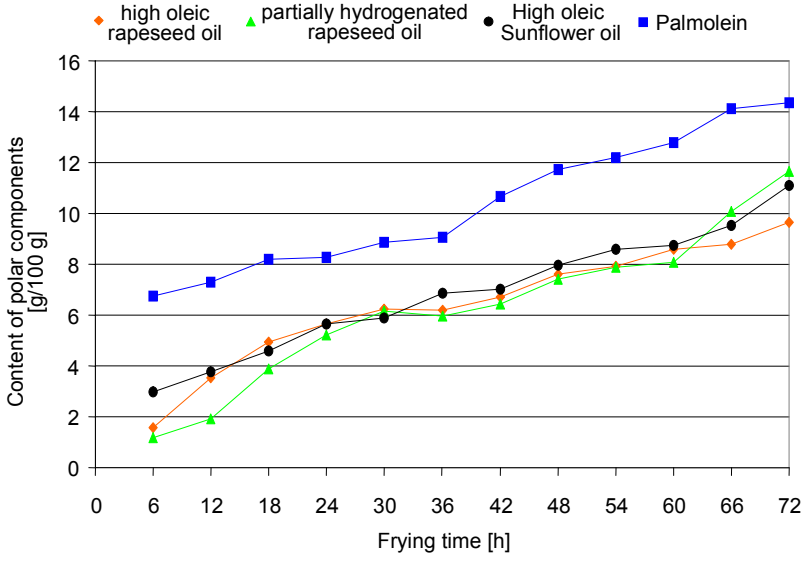

Fig. 2. Development of polar compounds during a frying period of 72 h.

Table. 2 Summarized results, calculated from the most important parameters

Type of oil	Oligomer triglycerides	Polar compounds	Free fatty acids	Sensory evaluation (oil)	Sensory evaluation (French fries)	Total result
HOLL	2	3	4	1	1	2.2
HOSO	3	1	2	4	1	2.2
PHRO	1	2	1	4	4	2.4
PO	4	4	3	4	1	3.2

HOLL=high-oleic rapeseed oil; PHRO=partially hydrogenated rapeseed oil;
HOSO=high-oleic sunflower oil; PO= Palm olein

Conclusion

The investigation shows that the use of HOLL rapeseed oil is an interesting alternative to common used oils. Looking on the results of the frying experiment indicates that HOLL rapeseed oil came to better or at least comparable results with regard to the sensory evaluation and the chemical parameters used for the assessment of the oils and the products being fried.

From this it can be concluded that HOLL rapeseed oil fulfils most of the demands necessary for frying oils, because it has some advantages with regard to health aspects, shows a high oxidative stability and the oil results in high quality and tasty food. The oil meets the needs of nutrition, taste and functionality, because it is low in saturated fatty acids, contains no *trans*-fatty acids and is high in oleic acid. The amount of linolenic acid is only moderate. Additionally the oil shows a high frying stability comparable to common used frying oils and results in good flavour characteristics of the products being fried.

References

Anonymous (2000). Recommendations of the 3rd International Symposium on Deep Fat Frying - Optimal Operation. Eur. J. Lipid Sci. Technol. **102**, 594.

AOCS (1990). Official Methods and Recommended Practices of the American Oil Chemist's Society, Champaign, IL, USA.

DGF (2005). Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Precht D., Molkentin J. (1995). Trans fatty acids: Implications for health, analytical methods, incidence in edible fats and intake. Die Nahrung, **39**, 343-374.

Stender S., Dyerberg J., Holmer G., Ovesen L., Sandström B. (1995). The influence of *trans* fatty acids on health: A report from The Danish Nutrition Council. Clinical Science. **88**, 375-392.

Quality analysis of rapeseed oil crushed with different extruding-expansion pretreatments

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Abstract

We designed and assembled a high-oil extruding-expansion machine which used the dry mode and studied the pretreatment of expansion in oil processing. The interaction between extruder operation parameters and oil quality was obtained by determining the changes of AV (acid values) and POV (value of peroxide) in extruded oil under different extrusion processing conditions. The stability of directly extruded oil was mensurated. The effects of the extrusion system parameters on acid values of extruded oil in turn was temperature of die noddle, diameter of die nozzle, speed of screw, moisture of material, and the effects on POV in turn was moisture of material, temperature of die noddle, speed of screw, diameter of die nozzle. The stability of oxidation of the directly extracted extrusion-expansion oil is higher.

Key words: Extrusion-expansion, Rapeseed oil, Operation parameter, Fat quality

Introduction

At present, the extruders used in rapeseed oil production are using wet mode and high oil extruding-expansion machines. The prolonged process and the energy cost were high due to the spraying of vapor and the latter drying processing. We designed a high-oil extruding-expansion machine using dry mode for rapeseed oil processing, which simplified the processing procedure, reduced equipment investment and processing cost.

The effect on oil quality in extrusion had already been attracted people's interest. Bjorck found that the stability of fat acid in the course of extrusion was greatly reduced and also found that the extrusion could promote the union between fatty acid and the starch. Mustakas had reported that the value of peroxide increased in the extrusion along with the increasing of the extrusion temperature, the water content of the material (15%-30%) as the increasing of the time which materiel stayed in the extruder. Maga also observed that extrusion may lead to cistrans isomerism of unsaturated fatty acid. Daniels thought that the reduction of fatty acid oxidation sensitivity was the form of fat peroxide.

Fig 1 Dry-single-screw extruder for high-oil-content material developed in the experiment

However it was reported very little about the research of the extrusion oil quality that obtained directly, after the oil-bearing grops extruded. We studied the influence of extruder operation parameter on extrusion oil and fat quality through the extrusion oil quality analysis which obtained to the orthogonal experiment, which is useful to determine parameters in rapeseed extruding-expansion pretreatment processing.

Material and method

The moisture of the rapeseed was 6.622% and the oil content was 40.102%. The thousand-seed weight was 3.13g and average seed diameter was 1.61 mm. After the smashing the average grain diameter was 0.85mm. The unshelled entire grain ratio was 2-7%.

The speed of screw was stepless adjustable in the range of 0-1200r/min (Figure 1). The sleeve temperature was continuously adjustable from 0 to 300°C and with the temperature number to reveal the measuring appiance closed loop automatic control system. Extruder nib aperture having steps adjustable and the productivity was 40-45kg/h.

The rapeseed extrusion-expansion pretreatment processing was:

The rapeseed→cleaning up→smashing→expanding→extracting→extracting oil
↘ Extrusion oil

The fatty acid value of oil squeezed out from extruder was determined using a method described in GB/T 5530-85 and

the fat peroxide value was determined using the sodium hyposulfite titrimetric method.

The parameters of extruder operation were listed in Table 1. The four factors and five levels were designed using tow orthogonal revolving combination to give 36 groups. Regression analysis was performed using the Reda software and found that the effect rules between influence of extrusion oil quality and the extrusion system operation parameter.

Table 1 Experimental variables and levels

	Nib diameter ϕ x_1 (mm)	Nib temperature T x_2 (°C)	Speed of screw n x_3 (r/min)	Moisture of material w x_4 (%)
-2	8	85	25	6.6
-1	10	95	35	8.6
0	12	105	45	10.6
1	14	115	55	12.6
2	16	125	65	14.6

Consequence and analysis:

Arranged the experiment of extruding-expansion rapeseed according to Table 2 and examined target for rapeseed extrusion fat acid value y1 and peroxide value y2. After disposing experiment data by Rade software package, obtained effect rules by each operation parameter of extruder to it. The regression equation was:

$$y_1=3.97+0.53x_1+0.50x_2-0.11x_3+0.33x_4+0.05x_1^2+1.16x_1x_2-0.50-0.25x_2^2-0.58x_2x_3-0.20x_2x_4-0.09x_3x_4-0.01x_4^2$$

$$y_2=2.69+0.14x_1-0.11x_2-0.10x_3+0.20x_4-0.12x_1^2+0.15x_1x_2-0.14x_1x_3-0.43x_1x_4+0.24x_2^2+0.42x_2x_3-0.19x_2x_4+0.01x_3^2-0.32x_3x_4-0.39x_4^2$$

With the test results and regression equation which were obtained according to Table 2,the factor contribution ratio of the extrusion system parameter was listed in table 3.The order of the effects of the extrusion system parameters on acid value of extruded oil in turn was:nib temperature x2(factor contribution ratio 1.75),nib diameter x1(factor contribution ratio 1.54),speed of screw x3(factor contribution ratio 0.66),moisture of material x4(factor contribution ratio 0.41); The order of the effects of the extrusion system parameters on POV of extruded oil in turn was: moisture of material x4(factor contribution ratio 2.09), nib temperature x2(factor contribution ratio1.15), speed of screw x3(factor contribution ratio 0.74), nib diameter x1(factor contribution ratio 0.41). Obviously to the acid value of the extrusion oil, the nib temperature and the diameter were playing the critical role. But the influence of moisture of material on POV of the extruded oil was far bigger than the nib temperature on it.

Tab.3 The primary and secondary turn of the effects of every factor

Review index	Parameter			
	Nib diameter x_1	Nib temperature x_2	Speed of screw x_3	Moisture of material x_4
Acid value Y1	1.54	1.75	0.66	0.14
POV Y2	0.41	1.15	0.74	2.09

Under the superior parameter combined condition (ϕ=10mm,T=105°C,n=60r/min,w=6.6%) single factor analysis result to the equation was as shown in Fig. 2.

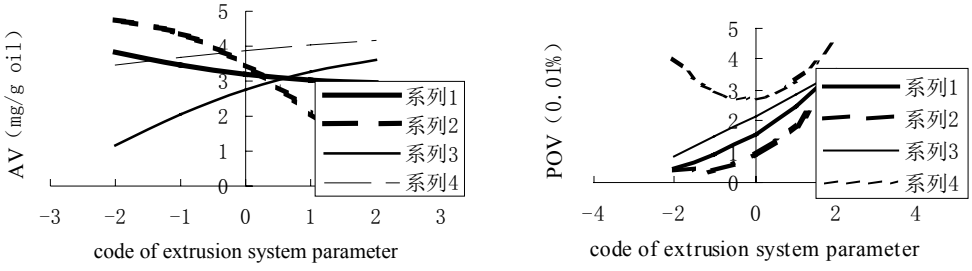
Tab2 Experiments scheme and results of characteristics of extrusion oil

Test number	x_1	x_2	x_3	x_4	AV y_1 (mg/goil)	POV y_2 (0.01%)	Test number	x_1	x_2	x_3	x_4	AV y_1 (mg/goil)	POV y_2 (0.01%)
1	1	1	1	1	3.87	2.72	19	0	2	0	0	3.46	3.07
2	1	1	1	-1	4.34	3.82	20	0	-2	0	0	3.98	3.83
3	1	1	-1	1	7.90	2.66	21	0	0	2	0	5.13	1.7
4	1	1	-1	-1	7.56	3.49	22	0	0	-2	0	3.54	3.34
5	1	-1	1	1	2.54	2.66	23	0	0	0	2	5.60	1.15
6	1	-1	1	-1	1.54	2.77	24	0	0	0	-2	3.69	0.69
7	1	-1	-1	1	2.46	3.56	25	0	0	0	0	4.13	1.20
8	1	-1	-1	-1	1.26	3.00	26	0	0	0	0	5.63	3.56
9	-1	1	1	1	2.43	3.54	27	0	0	0	0	4.58	3.03
10	-1	1	1	-1	2.06	2.65	28	0	0	0	0	4.13	3.33
11	-1	1	-1	1	2.51	2.14	29	0	0	0	0	2.14	2.81
12	-1	1	-1	-1	2.26	0.69	30	0	0	0	0	2.31	2.29
13	-1	-1	1	1	3.54	2.08	31	0	0	0	0	3.99	3.33
14	-1	-1	1	-1	3.20	2.38	32	0	0	0	0	2.25	2.38
15	-1	-1	-1	1	3.22	4.82	33	0	0	0	0	5.47	2.53
16	-1	-1	-1	-1	2.06	1.48	34	0	0	0	0	4.26	1.98
17	2	0	0	0	5.55	2.55	35	0	0	0	0	3.86	3.48
18	-2	0	0	0	4.25	3.34	36	0	0	0	0	4.86	2.32

Along with the nib temperature increasing,the acid value of the extrusion oil presented downside.The effect on the reduction to the acid value caused by high nib temperature increasing that caused the fat acid value was bigger than the low nib temperature increasing.It was because that along with the nib temperature increasing,materials flow rate in house increased

and caused the time that it stay in the high temperature sleeve reduce. Moreover as a result of heating temperature increasing,the fat viscosity reduced and also can urge the fat to be seperated from the material semifinished product in a short time. At this time,hot response time that became shortened holded the main status.The steatolysis function that caused by the temperature to the fat heating changed weakly. To this kind of effect on thecounter-flow oil, the nib temperature was higher,the effect was more remarkable.The nib diameter to the acid value of extruded oil presented the inverse correlation.when nib diameter enlarged, the friction and cutting of material which were received in house could change little, and the self-heating were few,therefore decomposition of fat being heated would reduce, and caused the acid value to reduce. Although the speed of screw of extruder increasing can cause the material flow rate to be quick,reduce dead time,and produce slightly affects to the fat decomposition.But along with rotational speed increasing,can have more intense cutting heat to the material,thus the fat decomposition speeded up and acid value heightened. This phenomenon that with the speed of screw raising caused the fat acid value to increase was more obvious when it was low speed of screw.The relation between the material moisture content and the acid value had certain effect.the high moisture content could promote the fat hydrolitic reaction to occur,so acid value increased.Because the time the material stayed in house was short,thus the scope of the acid value changing caused by the moisture content increasing was not to be big,and was also weaker for importance to effect on the acid value.

We discovered from this that in order to reduce the acid value of the extrusion,should choose the processing parameter of the high nib temperature,the big nib diameter,the low speed of screw and the low material water content as far as possible to carry on the production.



series 1- nib diameter of extruder, series 2-nib temperature of extruder, series 3-speed of screw, series 4-moisture of material

Fig. 2 Relationship between character of and extrusion system parameters

The effect on the material moisture content increasing to the POV was rising first,then to fall.As medium in chemical reaction, along with moisture content increasing, oxidation occurred in raw material intensified, peroxide in fat increased and peroxide value increased. but after the moisture content increased to a certain degree,with the moisture content continued to increase, it could displayed the characteristic that the dead time was short staying in house. Therefore the fat oxygenolysis changed weak and peroxide value reduced.When it was low nib temperature, the fat peroxide value growed slowly. Along with the nib temperature increasing,the peroxide value ingcreasing intensified.It was as a result of along with the temperature increasing,the speed of fat being heated oxygenilysis increased. The temperature was higher,the peroxide accumulation were more. Therefore the fat peroxide value increased remarkablely.The speed of screw and the extrusion oil peroxide value presented nearly the linear correlation.The influence mechanism of the enhancement of rotational speed and increasing of the temperature was the same to the fat.The rotational speed enhancing could cause the cutting hot of the material increase.This kind of function was evener and thorougher than it which the nib diameter to peroxide of extrusive oil also presented the correlation.This had direct correlation with the change of the nib diameter to the change of themeterial flow rate in house.The nib diameter was small,and the material flowing was blocked,then accumulation material semifinished product in house increased.The gun pressure which was observed from press chamber stengthened. Therefore it urged the fat to be separated from the material semifinished product as soon as possible.The probability that the fat was oxidized reduced the peroxide accumulation in fat were few.

From the single factor analysis chart between the extrusion oil peroxide value and the extrusion system parameter,we may see that in order to reduce the extrusion oil the peroxide value,we should choose the extrusion operating condition of the low or high material water content, the low nib temperature,the low speed of screw and the low nib diameter.

The index that peroxide value of the fat acted the determination initial oxidation was not only may express the fat oxidation degree in a certain extent,but also had high relevancy between the peroxide value after storage and the sense smell fetid odor and the flavor mark.In order to review the oxidation stability of the direct extrusion oil,this experiment observed the change of the peroxide value of the rapeseed extrusion oil stored in the oven at 60°C,and the peroxide value of the rapeseed extrusion oil stored,as shown in Figure 3.

In ordinary situation, when the peroxide value was higher than 0.1%,the fat appeared unhappy peppery saver and other irritant smell.In this experimental observation for 14 days time,the total peroxide content in fat had not appeared the obvious sudden change phenomenon.In the last stage of observation, peroxide value in fat was only 0.06% and was lower than the rapeseed high-quality cooking oil by 0.127% profession standard (ZBX14211-87).Not only the stability of the fat and the food including the fat had related with the existence of the unsaturated fatty acid and the resistant oxidation,but also had close correlation on way which produced oil and fat known from determination reslut of the resistant oxidation value change,rapeseed after the direct extrusion oil obtained had higher oxidation stability.

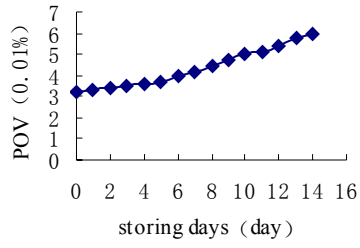


Fig 3 Storage Stability of Extruded Oil

T Wang compared the fat stability in the extrusion with unextrusion big wheat flour.The result indicated that the fat stability in the extrusion big wheat flour was lower than the extrusion sample. The proportion was lower of the unsaturated fatty acid and the saturated fatty acid in the unextrusion sample.At the same time,content of conjugation diolefine was high.T Wang proposed the extrusion-expansion desensitize activity of the lipase and the fat oxgenase, thus enhanced the stability of the soybean fat.this accorded with our test result.

4. Conclusion

The order of the effects of the extrusion system parameters on acid values of extruded oil in turn was temperature of die noddle, diameter of die nozzle, speed of screw, moisture of material,
The effects on POV in turn was, moisture of material, temperature of die nozzle, speed of screw, diameter of die nozzle.
The stability of oxidation of the directly extracted extrusion-expansion oil is higher.

Refernces

1. Liu Dachuan,Zhang Lin,Zhou Guang Sheeding the rapeseed skin,the extrusion-expansion,the new processing of extracting oil Chinese fat 2003, (1):p17
2. Zhang Fangpin,Liu Wensheng Practice on the high oil materiel with directly expanding and extracting Chinese fat 2001,26(2):p49
3. Shen Dechao,ZhangZhaoguo,Zhan Min and so on Study on the influence of the oil extraction rate of rapeseed by extrusion-expansion system parameter Agricultural enhineering journal 2004,20(6):p186
4. Zhang Min,Shen Dechao,Zhang Zhaoguo Experiment on the influence of the extrusion-expansion pretreatment to the character of the rapeseed material semifinished product. Argricultural machincal journal 2006,37(3):72-75
5. D K Bredeson Mechanical oil extraction J.Am.Oil Chem.Soc 1983,60(2):P163
6. S K Rao, W E Artz Effect of Extrusion on Lipid Oxidation J.Food.Sci 1989,54(6):P1580
7. S F O'Keefe, V A Wiley and D A Knauft Comparison of oxidative stability of high- and normal-oleic peanut oils J.Am.Oil Chem.Soc 1993,70(5):P489
8. T Wang, L A Johnson Natural refining of extruded-expelled soybean oils having various fatty acid compositions J.Am.Oil Chem.Soc 2001,78(5):P461
9. T Wang, L A Johnson Survey of soybean oils and meal qualities produced by different processes J.Am.Oil Chem.Soc 2001,78(3):P331

The reseach on the double low rapeseed protein concentrated by a new preparation method and its functional properties

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Abstract

The extraction condition was optimized by Response Surface Methodology. Results show that 70% ethanol water solution at 60°C, with the rate of 8.85:1, washing for six times and 20 minutes each, were optimum for defatted double-low rapeseed. By this way, the rapeseed protein concentrate reached 62.48% in protein content and was light in color, bland in taste. In the product, glucosinolation can be totally taken off and phytic acid descended by 60%. Its nitrogen solubility, water sorption, oil sorption, emulsifiability and foamability were studied and improved also. The product was fitting to be used as food additive.

Key words: double low rapeseed protein concentrate, protein, phytic acid, response surface methodology, the functional properties.

Introduction

Rapeseed, one of the most important oilseed crops cultivated in the world is becoming of increasing interest as a source of edible protein. Rapeseeds contain 35~47% of protein, and hence defatted rapeseed meal may constitute a good source of proteins for humans. Its amino acid composition is well-balanced in regard to FAO requirements.Moreover, oilseed protein is rich in sulfue-containing amino acids and lysine which are generally limited in legumes and cereals.

The preparation for the rapeseed protein concentrate is the way of extracting glucosinolation, phytic acid, tannin and so on from defatted rapeseed rapeseed, removing non soluble protein, causing the protein content to concentrate approach 65% in rapeseed protein product. Profits from other oil protein sources to take out phytic acid, the dissolution of characteristic difference between protein and phytic acid were used to separate them. The glucosinolation is soluble in water and the polyphenol can be in alcoholate.

In the present paper, the double low defatted rapeseed meal was used as material for the rapeseed protein concentrated. The ethanol water solution was chose as solvent and RSM was designed to optimize the parameter in experiment. It was also discussed the functional properties of the rapeseed protein product. The results and data could provide a theoretical basis for extensive application of concentrated rapeseed protein in food industry.

Materrials and methods

The double low defatted rapeseed meal: After the double low rapeseed cleaning up, the rapeseed's wetness was adjusted to 6% in the drying oven and the drying oven's temperature was controlled under 45°C to prevent protein denaturation. Then the rapeseed was pelled off by the rice huller, defatted by ether for 48 hours, and smashed.

Table 1 The material's mainly composition

Composition	The double low defatted rapeseed
Crude protein(%)	37.86
Fibre (%)	5.37
Fat content (%)	0.30
Wetness (%)	5.31
Tannin (%)	1.21
Phytic acid (mg/g)	10.38
Iso thiocyante (mg/g)	0.80
Oxazolinethioketone (mg/g)	2.39

All chemicals incluing ethanol, ether were of analytical grade.

The content of protein determination: The content of protein was determined according to the micro-Kheldahl method. Crude protein content was calculated using a convertion factor of 6.25.

The phytic acid's content determination: The phytic acid's content was determined according to trichloroacetic acid(TCA) method.

Optimization of preparation conditions: The double low rapeseed protein concentrate was preparated by 70% ethanol water solution. A three-factor central composite design was employed to examine the response, the content of protein and phytic acid's content as changed with the independent variables, the rate of solution and defatted rapeseed(X1), washing times(X2) and how many mintues of each time(X3). A quadratic polynomial regression model was assumed for predicting the

response. Every factor (code X1 to X3) had three levels corresponding to three code values. There were totally 15 independent experiments. In every experiment, levels of the factors were arranged according to table 2. The model proposed was described in table 3. Experimental data were analyzed for response surface regression for a quadratic polynomial model using SAS software.

Table 2 Design of factors and levels in experiment

Factor	Code	Code value	Level
The rate	X_1	+1	9:1
		0	8:1
		-1	7:1
Washing times	X_2	+1	7
		0	6
		-1	5
Minutes of each time	X_3	+1	30
		0	20
		-1	10

Table 3 Different levels of factors arranged in experiments

Test number	Code value of experiment		
	X_1	X_2	X_3
1	-1	-1	0
2	-1	0	-1
3	-1	0	+1
4	-1	+1	0
5	0	-1	-1
6	0	-1	+1
7	0	+1	-1
8	0	+1	+1
9	+1	-1	0
10	+1	0	-1
11	+1	0	+1
12	+1	+1	0
13	0	0	0
14	0	0	0
15	0	0	0

Amino-acid analysis: Amino-acid analysis of HCl-hydrolyzed samples was carried out an automated Beckman instrument. This work was completed by the amino-acid analysis service of the Oil Institute of the Chingese Academy of Agricultural Sciences(CAAS). All amino acid data were corrected for 100% recovery.

Results

Optimization of technology for the double low rapeseed protein concentrate's preparation:Results of 15 experiments were shown in table 4. Content of protein and phytic acid were used as response values in analysis of response surface regression(RSREG). The equation $Y= a_0+ a_1X_1+ a_2X_2+a_3X_3+a_{11}X_1^2+ a_{22}X_2^2+a_{33}X_3^2+a_{12}X_1X_2+ a_{13}X_1X_3+ a_{23}X_2X_3$ was used as regression model. The procedure RSREG of SAS also gave values of paramrter estimated (table 5) and predicted values of the equation (table 6).

Table 4 The content of protein and phytic acid of 15 experiments

Test number	Protein(%)	Phytic acid(mg/g)
1	57.77	9.33
2	59.40	10.00
3	52.64	11.00
4	59.72	10.33
5	57.92	10.50
6	58.15	9.16
7	60.38	9.83
8	59.50	7.33
9	58.05	6.00
10	58.11	6.83
11	57.68	6.53
12	55.88	7.17
13	61.78	6.05
14	62.51	5.93
15	62.56	6.07

Table 5 Parameters estimated by regression model

Parameters	Protein	Phytic acid
a_0	62.28	6.02
a_1	0.024	-1.52
a_2	0.45	-0.04
a_3	-0.98	-0.14
a_{11}	-3.23	0.54
a_{22}	-1.20	1.65
a_{33}	-2.10	1.54
a_{12}	-1.03	0.04
a_{13}	1.58	-0.83
a_{23}	-0.28	-0.29

Table 6 Predicted values of regression model

Response values	The rate	Washing times	Minutes of each time	Calculated value	The type
Protein (%)	7.89	6.27	17.1	62.49	max
Phytic acid (mg/g)	9.82	6.04	25.4	4.59	min

Variance analysis of regression equation was conducted(table 7,8). F value of the model was bigger than $f_{0.05}(9,5)$. R^2 was 0.973 and 0.990, which showed that linear relationship between dependent variable and whole indepengent variables was significantly distinct.

Table 7 Variance analysis of regression equations of the protein's content

Variance source	Degree of freedom	The protein's content		
		Sum of square	Mean square	F value
Model	9	77.56	8.62	9.69*
Error	5	4.44	0.89	
Correct total	14	82.00		
Linearly dependent coefficient		R^2=0.973		

Table 8 Variance analysis of regression equations of the phytic acid's content

Variance source	Degree of freedom	The protein's content		
		Sum of square	Mean square	F value
Model	9	39.45	4.38	26.56**
Error	5	0.83	0.17	
Correct total	14	40.28		
Linearly dependent coefficient		R^2=0.990		

Figure 1and 2 were response surface diagrams of the protein and phytic acid's content.

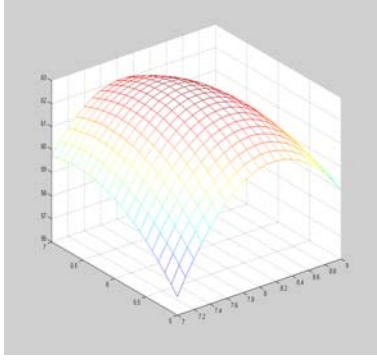

Figure 1 (1) :X-washing times Y-the rate Z-protein minutes of each time=17.1min

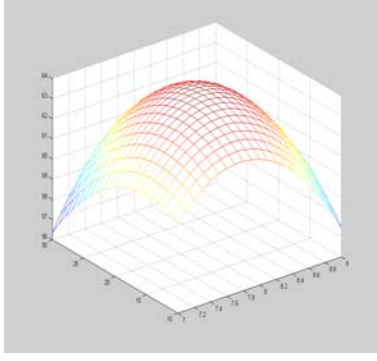

Figure 1 (2) :X- minutes of each time Y- the rate Z- protein washing times =6.27

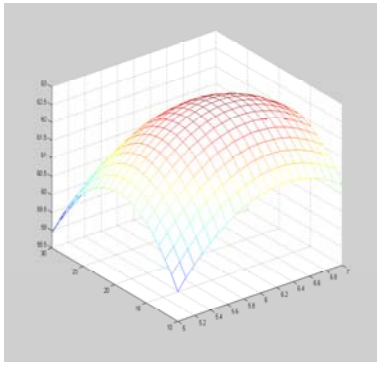

Figure 1(3): X- minutes of each time Y- washing times Z- protein the rate =7.89

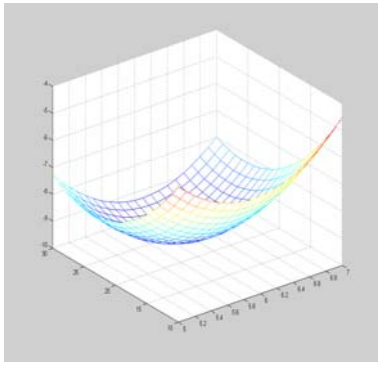

Figure 2(1):X- minutes of each time Y- washing times Z-phytic acid the rate =9.82

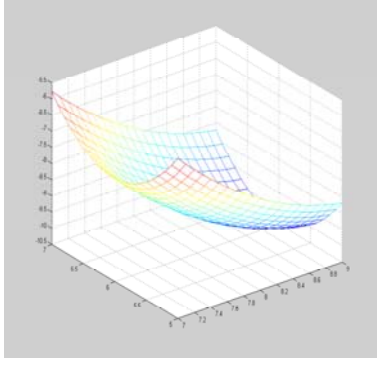

Figure 2 (2) :X- washing times Y- the rate Z- phytic acid minutes of each time =25.4min

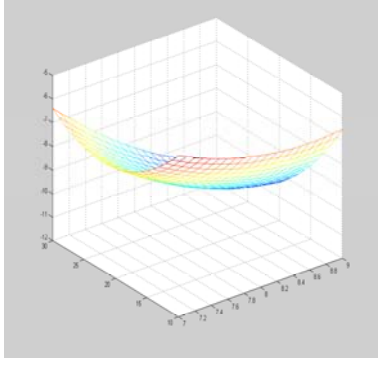

Figure 2 (3) :X- minutes of each time Y- the rate Z- phytic acid washing times =6.04

Discussion

Considering the interaction of all the variables, the optimum conditions for the preparation of double low rapeseed peotein concentrate can be calculated by the assumed equation as follows:70% ethanol water solution at 60°C, with the rate of 8.85:1, washing for six times and 20 minutes each.

By this way, the double low rapeseed protein concentrate reached 62.48% in protein content and was light in color, bland in taste. In the product, glucosinolation can be totally taken off and phytic acid descended by 60%. The 61% of the material can be gained.

The functional properties of double low rapeseed protein concentrate:The functional properties incluing solubility, water sorption, oil sorption, emulsifiability, foamability and so on can have the influence on the physics or chemical property to food quality. In recent years, protein products in food application are considered about not only its nutrition but also the physico-chemical properties. Therefore,the resrch on the functional properties of the double low rapeseed protein concentrate is extremely essential. The experiments were compared the functional properties of the double low rapeseed protein concentrate(RPC) and the soybean protein concentrate(SPC).(Table9,Figure3,4,5,6)

Table 9 The solubility of the RPC and SPC(pH=7.0)

Product	Soluble protein(%)	Nitrogen solubility(NSI,%)
RPC	2.12	3.37
SPC	3.47	5.10

Results showed the RPC's nitrogen solubility was lower for the protein denaturation, the RPC's water sorption and oil sorption were higher than SPC. But the RPC's emulsifiabilityand foamability were not as good as the SPC.

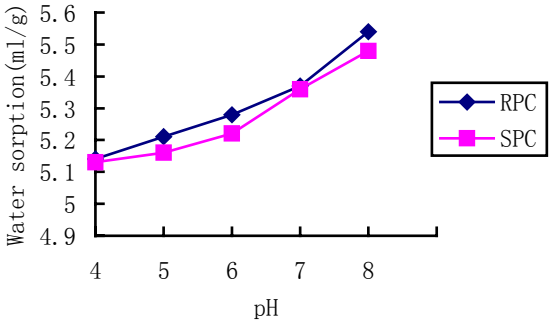

Figure 3 The water sorption of protein products

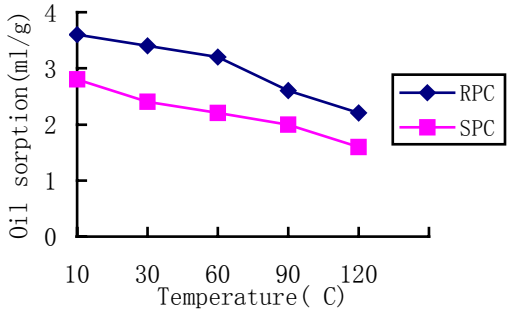

Figure 4 The oil sorption of protein products

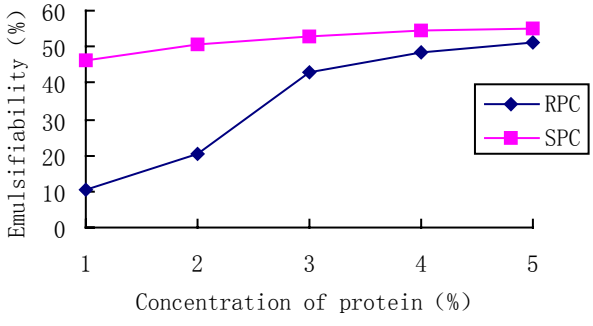

Figure 5 The emulsifiability of protein products

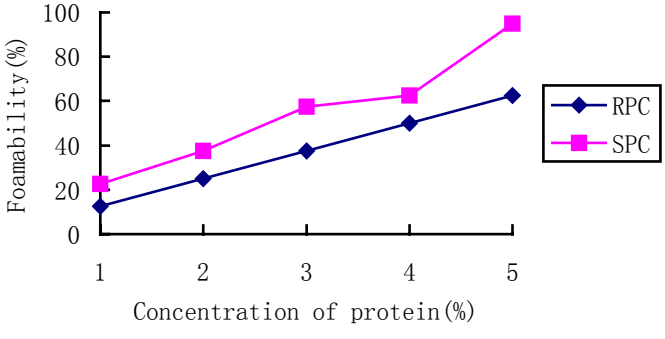

Figure 6 The foamability of protein products

Conclusions

The double low rapeseed protein concentrate was prepared by 70% ethanol water solution. The optimum conditions were established by RSM. These parameters included temperature: 60°C, with the rate of 8.85:1, washing for six times

and 20 minutes each. By this way, the rapeseed protein concentrate reached 62.48% in protein content and was light in color, bland in taste. In the product, glucosinolation can be totally taken off and phytic acid descended by 60%. The 61% of the material can be gained.

Table 10 The product's mainly composition

Composition	The double low rapeseed protein concentrate
Wetness(%)	7.24
Crude protein (%)	62.48
Fat content (%)	0.28
Fibre (%)	6.73
Ash content (%)	4.08
Tannin (%)	0.130
Phytic acid (mg/g)	4.62
Glucosinolation (mg/g)	not detected

Table 11 The product's composition of amino-acid

Amino-acid	Content(g/100g)	Amino-acid	Content(g/100g)
Aspartic acid	8.87	Methionine	1.55
Threonine	4.43	Isoleucine	4.06
Serine	4.13	Leucine	5.32
Glutamic acid	19.74	Tydroxyproline	2.90
Glycine	4.21	Phenylalanine	3.99
Alanine	4.39	Histidine	1.86
Valine	4.35	Lysine	1.83
		Arginine	2.40

The research on the functional properties of double low rapeseed protein concentrate showed the RPC's nitrogen solubility was lower for the protein denaturation, the RPC's water sorption and oil sorption were higher than SPC. But the RPC's emulsifiabilityand foamability were not as good as the SPC.

References

Bell J.M.,Jeffers,H.F..(1976).Varialility in the chemical composition of rapeseed meal.Can.J.Anim.Sci. **56,**269 – 273

Bell,J.M.,M.O.Keith.(1991).A survey of variation in the chemical composition of commercial canola meal produced in western Canadian crushing plants.Can.J.Anim.Sci. **71**,469 -480

Bell,J.M.Factors.(1993). Affecting the nutritional value of canola meal:a rewiew.Can.J.Anim.Sci. **73**,679-697

Chajuss D.(2001). Soy protein concentrate: processing,.Properties and prospects. **12**:,1176 - 1180.

Karnofsky G. (1985).Design of oilseed extractors: multicomponent ex traction. J. Am. Oil Chem. Soc.**63**, 1015 - 1016.

DeClercq,D.R.,J.K.Daun,et al. (1996).Quality of Western Canadian Canola.Crop bulletin No.230.ISSN 0836 - 1657. Grain ResearchLaboratory, Canadian Grain Commission.Winnipeg.MB

Gu Yusing, Hua Yufei, Liu Fuguang.(1997). Optimization of Alcohol Leaching Process for Soy Protein Concentrate.China Oils and Fats. **22**,12-15

Hancock J D.(1990). Effects of alcohol extraction and heat treatment on the utilisation of soyabean protein by growing rats and pigs. J. Sci. Food Agric. **52**, 193 - 205.

Robert,l.C.(1990). Protein from double-zero Rapeseed,J.Agric,Food chem. **36**,690-694

Simbaya J,Slominski B A,Rakow G,et al.(1995).Quality characteristics of yellow -seeded brassica seed meal:protein,carbohydrates,and dietary fiber components.J Agri Food Chem. **36,**2062 -2066

Yu Huamin. (1989). The Method of Rapeseed' s Detoxification. China Oils and Fats. **14**,51-53

Yu Ying, An Tingshi, Luo Chaozhong.(1994). Improvement of analytical methods for total glucosinolate contents in rapeseed,. China Oil Crop.**15**,52-54

Storage of rapeseed — an important aspect for the production of high quality native rapeseed oil

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Abstract

Native rapeseed oils become more and more important in Germany, but also in other countries. The problem of such oils is that the quality on the market is very heterogeneous, which makes the buying decision for the consumer difficult. The paper presents results of a research project showing that the most important period for the production of native rapeseed oil is not the oil processing, but the time between harvest and processing, with pre-treatment of the seeds and storage. Storage of the seeds with 9 % moisture as common used in large facilities is not suitable for the production of high-quality native rapeseed oil, because of the development of negative sensory sensations. Also foreign matter such as foreign or broken seeds, pods or stems strongly affects the quality of the resulting oil.

Key words: Oilseed rape, pre-treatment, quality, sensory quality, storage

Introduction

Since several years in Germany, but also in other countries additionally to refined rapeseed oil native rapeseed oil as edible oil is available on the market, according to the example of native olive oil. Reasons for this interest are the demand of the consumers for less processed food and the increasing desire for edible oils with a natural taste and smell according to the material it derives from. From a nutritional point of view both types of oil can be assessed as comparable, but nevertheless there are some differences which are important for the consumer and which result in differences for the demands on the initial seed quality. While large centralized facilities with extensive oil processing and following refining have the chance to improve oil quality independently on the seed quality, small decentralized facilities are strongly dependent on the quality of the seeds. The simple oil production by means of a screw press with sedimentation or filtration as only cleaning steps does not allow an improvement of the oil quality after processing (Fig. 1).

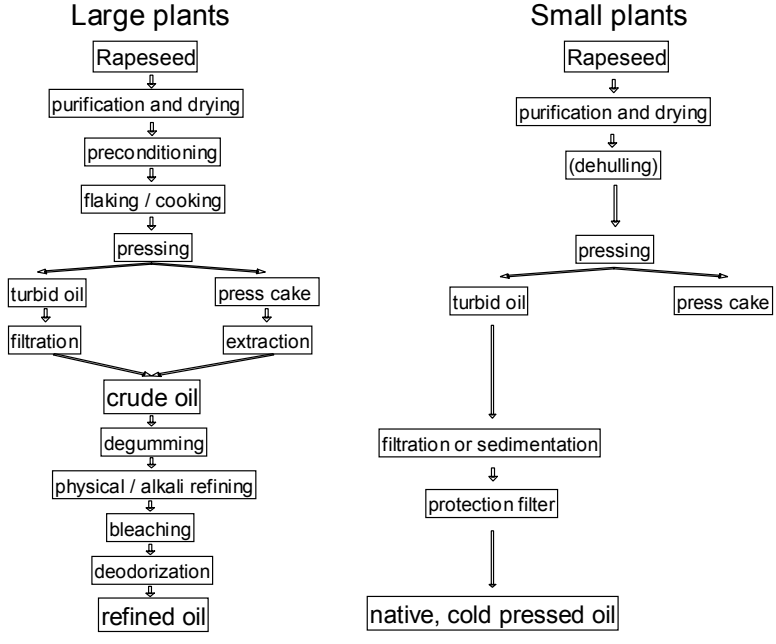

Fig. 1. Oil processing in large and small plants.

Why is it so difficult to produce high-quality native rapeseed oil?

One key point for the production of high quality native rapeseed oil is that it is absolutely necessary to maintain the good

quality of the seeds after harvest on a high level until processing. Looking on the market of native rapeseed oils in Germany makes clear the dilemma of this type of oil. The quality of the oils on the market and especially the sensory evaluation is very heterogeneous. While some oils have a typical *seed-like* and *nutty* taste and smell as expected by the consumer, several oils have to be characterized as unsuitable for human consumption, with sensory defects like *musty*, *fusty*, *yeast-like* or others.

Studying the composition of rapeseed points out why it is so difficult to handle oilseeds in general and rapeseed especially without losses of quality. In comparison to cereals with only 2% of fat, the fat content of rapeseed is about 45%. On one hand fat is very susceptible against oxidation and degradation products of the oxidative process are already noticeable in very low concentrations. On the other hand fat is an excellent carrier for aroma components, which retains and concentrates volatile compounds. This implicate that the high oil content of rapeseed acts as the sensory memory of the seed. Everything what results in an impairment of the sensory evaluation during pre-treatment and storage of the seeds up to the processing is memorized by the oil. In native, cold pressed oils these failures are noticeable directly by sensory evaluation.

The results of the market survey emphasize that the production of high-quality native rapeseed oil is very difficult and it must be taken into consideration that seeds harvested from the field are living organisms, which are strived for developing a new plant. Additionally, an optimal storage of the seed material is only possible, if the seeds stay alive during storage. Therefore before and during storage of the seeds producers of native rapeseed oil have to do the splits between reducing the respiration rate of the seeds and keeping the seeds alive.

Influence of the storage conditions

For this the seed management is most important for the production of high quality native rapeseed oil for human consumption. This includes a careful choice of the raw material and an appropriate pre-treatment of the seeds. The main problem during storage is moisture, because a certain amount of water is sufficient for the development of micro organisms, but also for the activity of enzymes. Both strongly affect the quality of the resulting oil. Sources of moisture are either the seeds themselves or especially foreign matter, such as broken or foreign seeds, pods or stems. With increasing amounts of moisture the respiration rate of the seeds increases and the result is the degradation of storage compounds like triglycerides, carbohydrates and proteins and the development of volatile and non-volatile degradation products such as free fatty acids or aroma-active compounds.

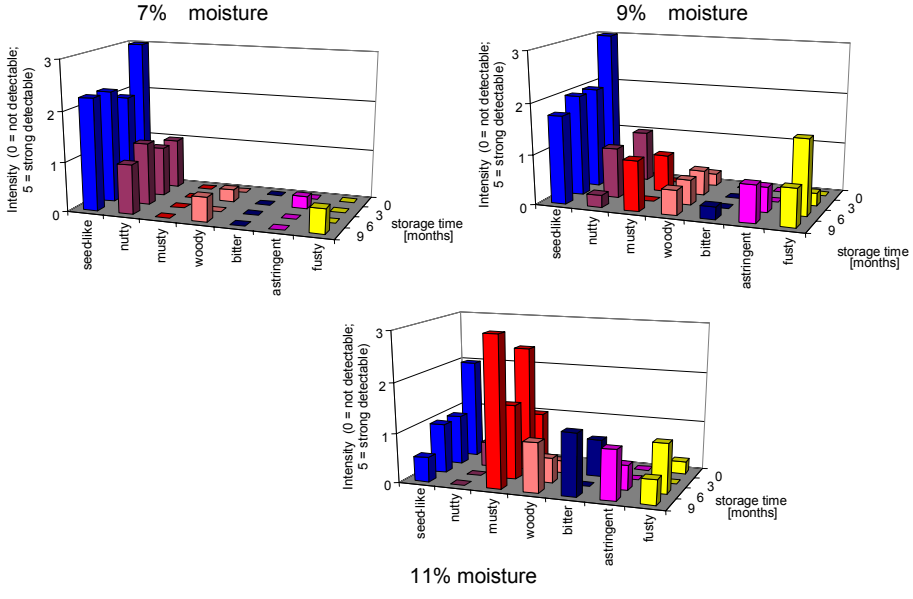

Fig. 2. Storage of rapeseed with different amounts of moisture over a period of nine months and sensory evaluation of the resulting oils.

Therefore it is necessary to dry the seed material after harvest carefully, if the moisture content is higher than 8 %. Figure 2 shows the results of a storage experiment with rapeseed containing different amounts of moisture. The seeds were stored over a period of 9 month and at appropriate dates seed material was pressed under defined conditions. The resulting oil was tasted by a trained sensory panel.

Seeds stored with a moisture content of 7% were stable over a period of nine months. The resulting oils showed no decrease of the positive sensory attributes *seed-like* or *nutty* and no development of negative attributes like *fusty* or *musty*. During storage of the seeds the resulting oil quality remained constant on a high level. The result is different, if the moisture content increases to 9%. In that case a clear decrease of the positive attributes and an increase of negative attributes occurred, making the resulting oils inedible after six months. Storage of rapeseed with 11% moisture resulted even in a faster development of negative attributes while positive sensations were only detectable within the first month of storage. The results of the experiment show that a limitation of the moisture content on 9% as used for large plants which have the possibility to improve oil quality by refining is not sufficient for producer of native rapeseed oil. Here a value between 7 and 8% has to be the aim. Additionally to the sensory quality of the oils, storage of rapeseed at 9% moisture or higher results in a continual

increase of free fatty acids during a storage period of 9 months. No change in the content of free fatty acids was detectable at moisture of 7% (Attenberger et al., 2005).

Influence of foreign matter

The existence of foreign matter is also critical. On one hand foreign matter consisting of broken seeds, foreign seeds, sprouted seeds, but also pods and other foreign materials influences the oil yield, since it presents noticeable lower oil contents. On the other hand oil quality is declined, because the content of free fatty acids and chlorophyll in sprouted, broken and foreign seeds, respectively, is much higher than in intact rapeseed. As long as the oil is protected by the intact seed hull and separated from the appropriate enzymes in compartments it is stable against hydrolysis into free fatty acids and oxidation reactions. Not until this protective hull is damaged during harvest or storage, or pest like mite or insects try to get to the ingredients these degradation reactions run. It is important to take into consideration that the number of micro organisms on foreign matter is up to 100 times higher than on intact rapeseed. In addition, especially foreign seeds contain distinctive higher amounts of moisture, which also pass on to rapeseed during storage. About 40% of the volume of stored rapeseeds consists of hollows, in which an appropriate relative humidity is adjusted, depending on the amount of seed moisture. This humidity set the speed of metabolism in the seeds and favours the growth of mould, yeast and bacteria on the seeds during the storage period.

Therefore higher amounts of foreign matter result in a significant impairment of the aroma and taste of the resulting rapeseed oil. Figure 3 shows that only 5% broken seeds in cleaned rapeseed lead within 6 days of storage at room temperature to *fusty* and *musty* aroma components, while foreign matter like stems, pods or foreign seeds results in *wood-like* and *straw-like* or also *fusty* and *musty* sensations, depending on the composition of the foreign matter (Attenberger et al., 2005). Such oils are not usable as food or for food preparations.

Hence a careful cleaning of the seed material after harvest before storage is very important due to the fact that already small amounts of foreign matter result in a notable deterioration of the oils concerning sensory evaluation and chemical parameters.

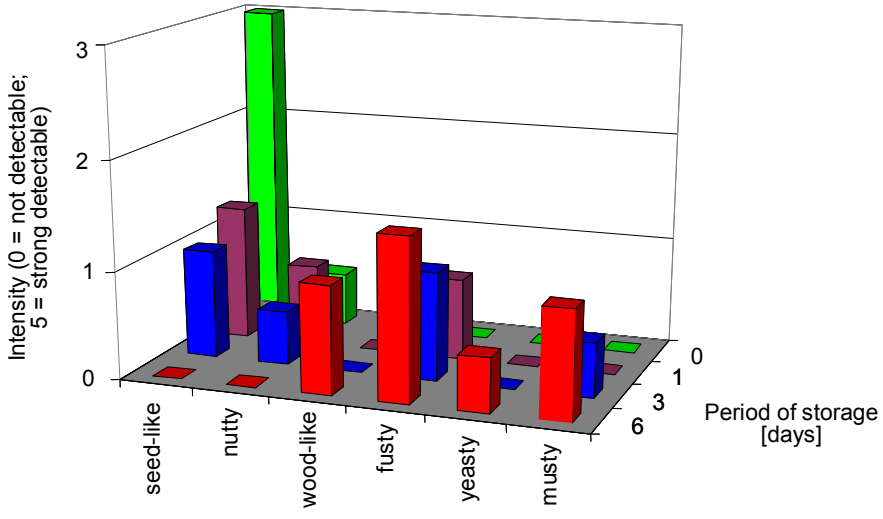

Fig. 3. Influence of 5% broken seeds in intact seeds during 6 days of storage at room temperature on the sensory evaluation of the resulting oil.

Conclusions

The main factor affecting the quality of native rapeseed oil is the period from harvest to processing and not the processing. Within this time the high oil content of the seeds acts as sensory memory and everything what was done with the seeds is coming back as taste and smell of the oil. Since producer of native rapeseed oils have no possibility to improve the quality of the oil during processing it is absolutely necessary that they take care on the quality of the raw material.

References

Attenberger A., Matthäus B., Brühl L., Remmele, E. (2005). Research into the influencing factors on the quality of cold pressed rapeseed oil used as edible oil and determination of a quality standard. Eigenverlag, Technologie- und Förderzentrum, Straubing, Germany, 84-91.

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Influence of spices on the quality of rapeseed oil during storage

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Abstract

The paper presents some results of an investigation dealing with the effect of flavouring of rapeseed oil with rosemary, thyme and basil on the oxidative stability, sensory evaluation and content of phenolic compounds of the oils. The oils were stored for 26 weeks at room temperature and 7 days at 50°C, respectively. The addition of spices had some effect on the oxidative stability, but the effect was not much pronounced at room temperature. Only after 22 weeks the oils without spices showed a stronger increase of the peroxide value than the flavoured oils. At 50°C the effects were clearer. Addition of rosemary and thyme resulted in only a small increase of the peroxide value and also the addition of basil reduced the increase of oxidation in comparison to oil without spices. The sensory quality of the oils changed during storage from an aroma typical for the used spice to a more astringent taste. During storage an increasing amount of volatile compounds from the spices were found in the oil. An increase of the total volatile compounds during storage was observed. Some of the phenolic compounds migrate from the spices into the oil, but the amount was small and the main phenolic compounds of spices were not found.

Key words: basil, flavouring, oxidative stability, phenolic compounds, rapeseed oil, rosemary, thyme

Introduction

Edible rapeseed oil became increasingly popular within the last years, because of the interesting fatty acid composition, which has some advantages from the nutritional point of view. For the marketing with edible oils it is necessary to present continuously new and innovative products to the consumers. In this context the flavouring of edible oils with spices for the preparation of salads and other foods occurred on the market. Some of these spices are known to contain higher amounts of effective antioxidant components, e. g. rosemary (Schwarz et al., 1992; Hopia et al., 1996; Nguyen et al., 1999). From this the aim of the work was to investigate the effect of spices on the quality of the oils during storage, especially in view of the oxidative state and the sensory quality.

Materials and Methods

Each, 1.5 g of dried material from rosemary, basil and thyme, respectively, purchased from a local supermarket was added to 200 mL of refined rapeseed oil. Several bottles of the oils flavoured with spices were stored at room temperature for 26 weeks and every two weeks at the beginning of the experiment, later every four weeks one bottle was taken and the oils were assessed with regard to the sensory quality. Additionally some chemical parameters describing the oxidative status of the oils were investigated. The content and the composition of phenolic compounds extracted from the oils were determined. Also a storage experiment at accelerated temperature over a period of 7 days at 50°C was carried out.

Table 1. Methods used for the evaluation of the status of the stored oils

No.	Parameter	Method	Reference
1	Anisidine value	DGF C-III 3b	DGF, 2005
2	Oxidative stability (Rancimat 120°C)	Cd 22-91	AOCS, 1990
3	Free fatty acids	DGF C-V 2	DGF, 2005
4	Tocopherol composition	DGF F-II 4	DGF, 2005
5	Peroxide value	DGF C-VI 6a (02)	DGF, 2005
6	Sensory assessment	modified DGF C-II 1 (97)	DGF, 2005
7	Volatile compounds	Dynamic Headspace-GC	Brühl and Fiebig, 2005
8	Content of phenolic compounds	HPLC	Mateos et al., 2001

Results

The results show that all types of spices used in this investigation were suitable to change the sensory sensation of the initial oils towards the taste and smell of the appropriate spice, because oil as a very good aroma carrier took in the aroma components of the spice. Shortly after addition of the spices the oils tasted and smelled accordingly to the appropriate spice. After 20 weeks of storage the aroma changed to more unpleasant sensations and became increasingly astringent. Figure 1 shows the increase of volatile compounds during storage of oil after addition of rosemary over a period of 6 days at 50°C. During this time the amount of volatile compounds migrated from the spice into the oil increased for about 50 times. A similar

result, but not as clear was also found for rosemary, basil and thyme stored at room temperature.

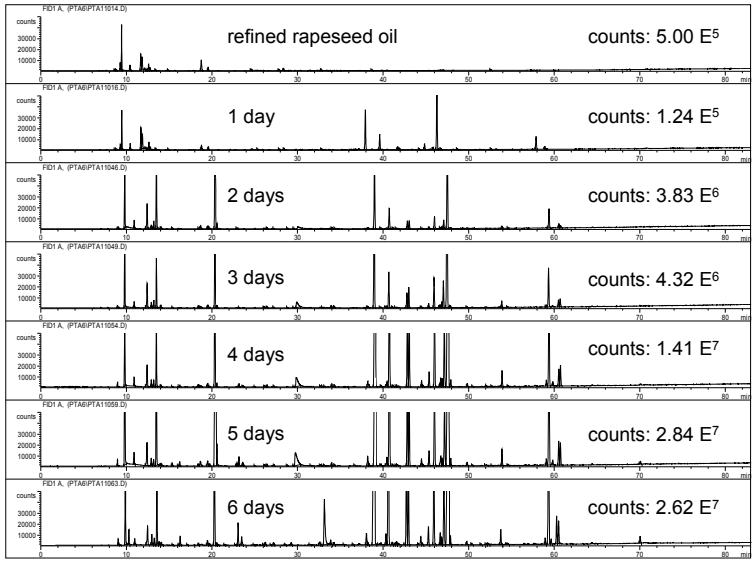

Fig. 1. Development of volatile compounds in rapeseed oil flavoured with rosemary stored at 50°C over a period of 6 days.

Only a small increase of the content of free fatty acids was observed for the oils stored with different spices, although the increase in oils flavoured with spices was higher in comparison to oils without addition of spices. But the differences between the oils were only significant for thyme ($p < 0.05$) (results not shown). One reason for a slightly faster formation of free fatty acids after addition of spices could be spice-own or microbial enzymes which are able to degraded triglycerides. Nevertheless for a high quality of the oil a microbial clean spice material is necessary, since otherwise a faster degradation of triglycerides and a faster formation of free fatty acids is to be expected.

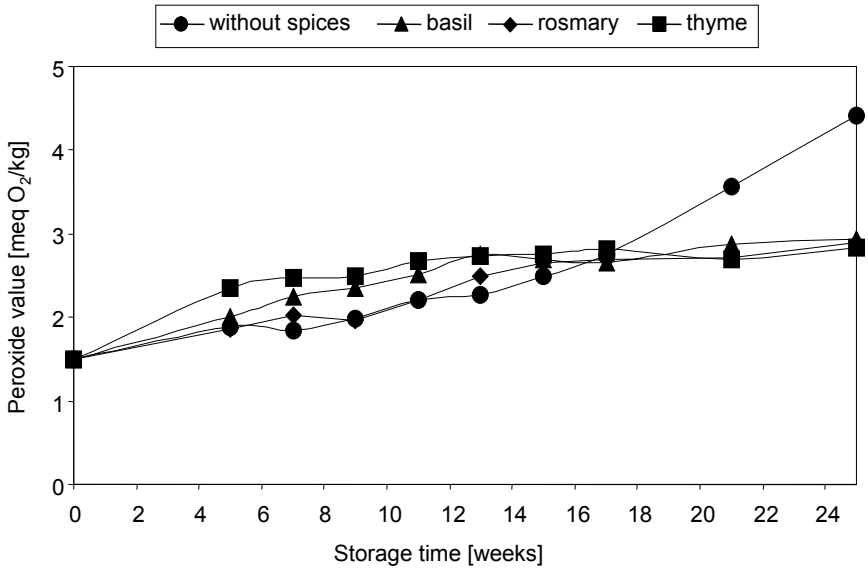

Fig. 2. Effect of basil, rosemary and thyme on the peroxide value of rapeseed oil during storage at 20 °C.

The influence on the oxidative state of oils enriched with spices during storage depends on one hand on the composition of the phenolic compounds of the spices and on the other hand on the migration of phenolic compounds from spices into the oil. Figure 2 shows the development of the peroxide value of oils enriched with rosemary, basil and thyme in comparison to oil without addition of spices. While the peroxide value of the oils enriched with spices showed only a weak increase of the peroxide value from 1.5 to 2.5 meq O_2/kg within 26 weeks of storage, oil without spices reached a peroxide value of 4.0 meq O_2/kg. This indicates a significant ($p < 0.05$) influence of the three tested spices on the oxidative stability of the oil during storage. The same effect was observed for measuring the oxidative stability by Rancimat at 120°C. By addition of spices to the oils the oxidative stability measured by Rancimat was enlarged (results not shown).

The differences between the different spices were unincisive regarding the formation of peroxides, but the effect was much clearer at higher storage temperature. Possibly, storage at higher temperatures enables an easier migration of compounds with an antioxidative activity from the spices into the oil. Using 50°C as storage temperature, the peroxide value of oil without addition of spices increased within 7 days to about 30 meq O_2/kg, while addition of spices resulted in a significant

improvement of the oxidative stability of the oils.

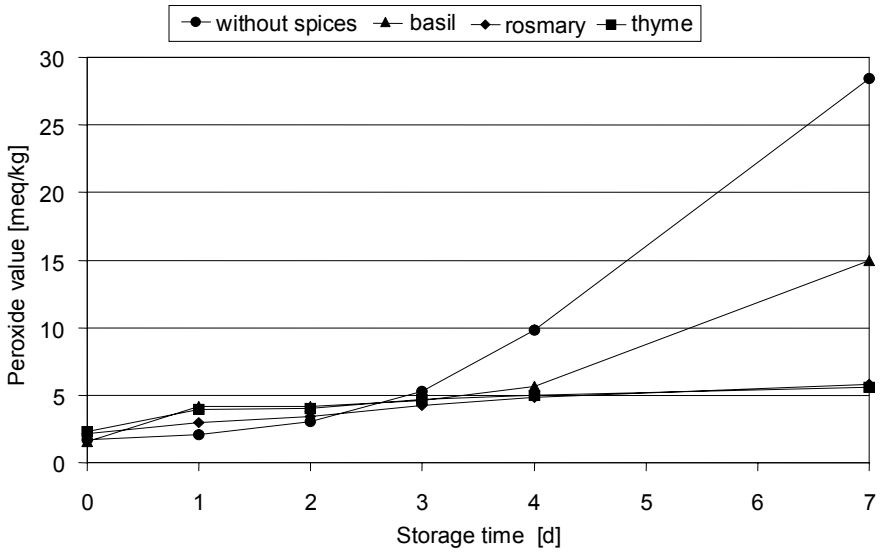

Fig. 3. Effect of basil, rosemary and thyme on the peroxide value of rapeseed oil during storage at 50 °C.

Flavouring with basil reduced the peroxide value for a half and the effect was much more pronounced by addition of rosemary and thyme, respectively. In that case the peroxide value increased only to about 5 meq O_2/kg. A similar result was found for the oxidative stability measured by Rancimat at 120°C which increased with increasing storage time for rosemary and thyme.

The content of tocopherols in rapeseed oil was not influenced by the addition of spices. During storage the content of tocopherols continual decreased, but no difference by the addition of spices in comparison to the samples without spices was observed (data not shown).

Looking on the migration of phenolic compounds from spices into oil it is obvious that only very small amounts of these compounds go into the oil. After 5 weeks only 10.3 and 58.2 mg/100 g oil were found in oil flavoured by basil and rosemary, respectively, in comparison to 7.4 mg/100 g of the initial oil. Figure 4 shows the HPLC-chromatograms of the phenolic compounds extracted from oils after storage with appropriate spices. It is obviously that the amount of some compounds increased as storage time went by. But interestingly rosmarinic acid, characteristic for and the main phenolic compound of spices was not found, while other substances already described as phenolic compounds of spices, luteolin and apigenin occurred in only small amounts. Although spices contain high amounts of phenolic compounds only a small amount is oil-soluble and migrates into the oil during storage. The main part of the phenolic compounds remains in the spices. Therefore the effect of the phenolic compounds on the oxidative stability is only small. Since the migration of phenolic compounds into the oil is improved at accelerated temperatures in that case spices can contribute to a higher oxidative stability.

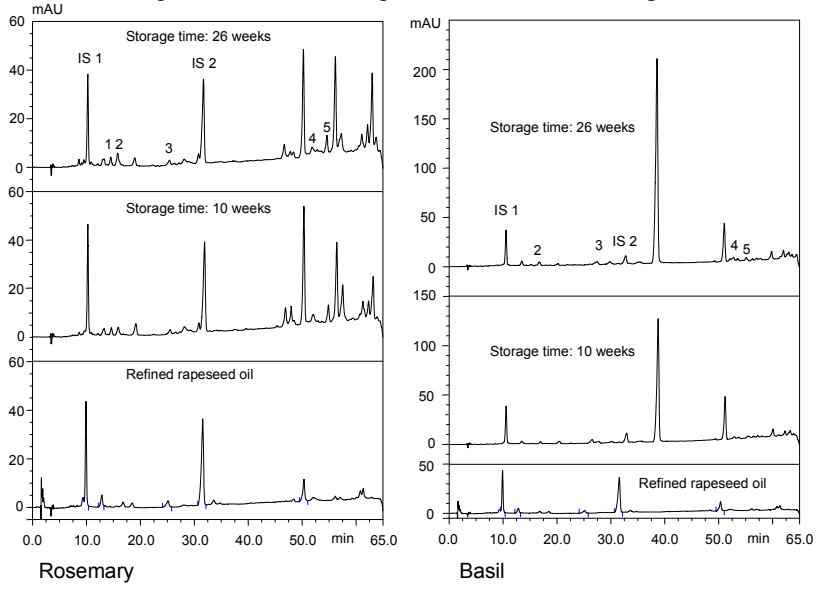

Fig. 4. HPLC chromatograms of phenolic compounds extracted from oils flavoured with rosemary and basil.

(IS 1 = p-hydroxyphenylacetic acid, IS 2 = o-coumaric acid, 1 = vanillin, 2 = vanillic acid, 3 = p-coumaric acid, 4 = luteolin, 5 = apigenin)

Conclusion

The improvement of the oxidative stability of oils by addition of and flavouring with spices is only small at room temperature and a little more pronounced at accelerated temperature, since the migration of phenolic compounds into the oil was improved by the higher temperature. The main effect of spices is the flavouring of the oil and not the improvement of the oxidative stability. During storage the amount of volatile compounds which migrate from spices into the oil increased significantly, but during long-term storage the taste of the oils changes to astringent sensations, which has to be taken into consideration.

References

AOCS (1990). Official Methods and Recommended Practices of the American Oil Chemist's Society, Champaign, IL, USA.

Brühl L., Fiebig H.-J. (2005) Assistance of dynamic headspace chromatography for panel sensory evaluation. Riv. Ital. Sost. Grasse. **82**, 291-297.

DGF (2005). Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Hopia A., Huang S.-W., Frankel E. (1996). Phenolic diterpenes from rosemary as antioxidants in linoleic acid, methyl linoleate and corn oil triglycerides. Food Chem. **57**, 57-59.

Mateos R., Espartero J. L., Trujillo M., Rios J. J, León-Camacho, M., Alcudia F., Cert, A. (2001). Determination of Phenols, Flavones, and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection. J. Agric. Food Chem., **49**, 2185-2192.

Nguyen H. T. T., Pokorny J., Korczak J. (1999). Antioxidant Activities of Rosemary and Sage Extracts in Rapeseed and Sunflower Oils. Czech, J. Food Sci. **17**, 121-126.

Schwarz K., Ternes W., Schmauderer E. (1992). Antioxidative constituents of Rosmarinus officinalis and Salvia officinalis. Part 3. Stability of phenolic diterpenes of rosemary extracts under thermal stress as required for technological processes. Z. Lebensm. Unters. Forsch. **195**, 104-107.

Research progress on the function and synthesis of phytosterol esters of fatty acids

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Abstract

Phvtosterol esters are kinds of novel serum cholesterol lowering functional food additives. A detailed introduction to progresses in the function and the synthesis of phvtosteryl esters, especially the enzymatic synthesis,are discussed in this paper in order offering consults for the application research of phytosterol ester.

Key words: phytosterol esters; phytosterols; function; synthesis; lipase

Introduction

Plant sterols (phytosterols) are sterols derived from plant sources, such as vegetable oils and cereals a. In plants, more than 40 sterols have been identified with β-sitosterol being the most abundant. Phytosterols are important, due to their recent recognition and application in the food and nutraceuticals industries as cholesterol lowering agents, which are known to have a hypocholesterolemic effect by lowering plasma total and low density lipoprotein (LDL) cholesterol levels without affecting plasma high density lipoprotein (HDL) cholesterol concentration(Pollak, 1953; Beveridge, 1964; Lees, 1977). The safety of phytosterols has been affirmed by government agencies such as the US Food and Drug Administration and the European Union Scientific Committee. Phytosterol is one of 10 function ingredients in the future, 2000 year FDA authorized foods added phytosterol or ester could use "healthy" label (Jones, 2000). 2004 year European Union committee passed the foods added phytosterol or esters of ARCHER DANIELS MIDLAND Company (ADM) and Unilever Company.

1 Function

1.1 Absorption of phytosterols and Toxicity studies

An early summary of phytosterol absorption by various animal species showed absorption ranging from 0% (rabbit) to 4% (rat), and 6% (human being) (Pollak, 1981). Salen et al (Salen, 1949) fed human sitosterol and estimated absorption at 1.5% to 5%. Ostlund et al (Ostlund,2002) studied the absorption of different phytosterols in human subjects and found out that sitosterol and campesterol were absorbed at levels of 0.512% ±0.038% and 1.89% ±0.27%, respectively. Salo et al. (Salo, 2002) thought the minimum amount of sterols and stanols required to produce a significant LDL cholesterol lowering effect is about 0.8~1.0 g/day. European Union recommended the quantity was 3g/d.

From Shipley et al (Shipley,1958) to Hepburn et al (Hepburn, 1999) the authors found no detectable effects on growth, serum proteins, blood urea nitrogen, or gross or microscopic appearance of any organ or tissue. No treatment-related changes were observed (Baker, 1999; Weststrate, 1999; Ayesh,1999; Sanders,2000; Wolfreys,2002; Lea,2004;) No indications of estrogenic effects were observed, nor were there effects on levels of reproductive hormones in female volunteers. Phytostanol esters are not genotoxic in in vitro gene mutation assays using mammalian or bacterial cells (Whittaker, 1999; Turnbull, 1999). To date, no evidence of toxicity or genotoxicity was found.

1.2 Cholesterol-lowering effects

Phytosterols have been widely studied for their hypocholesterolemic, anticarcinogenic, and other health effects. For more than 50 years now, phytosterols are known to have a hypocholesterolemic effect allowing the reduction of LDL (low-density lipoprotein) cholesterol in plasma whereas high-density lipoprotein (HDL) cholesterol concentration is not affected by their consumption. The cholesterol lowering mechanism of plant sterols is attributed to an inhibitory effect on cholesterol absorption due to the chemical structure similarities of phytosterols with cholesterol. Thousands of people were enrolled in clinical trials which showed the efficacy and safety of the preparation based on phytosterols. Exhaustive results compiled from recent studies are summarized in the following table 1:

2 Synthesis methods

Phytosterols have generated interest in the functional food industry as they have been shown to reduce the levels of "bad" serum low-density-lipoprotein (LDL) cholesterol in human. However, free sterols have limited interest owing to their low solubility and high melting point. Apparently, fat-soluble plant steryl esters are able to lower plasma cholesterol levels more efficiently than the corresponding homogenized crystalline plant sterol preparations. Therefore, fatty esters of sterols are generally preferred in food formulation. These fatty esters sterols can be produced by chemical esterification and lipase-catalyzed esterification.

2.1 chemical esterification

The reaction by chemical esterification is faster than by lipase-catalyzed method. Phytosterol esters of FAs are presently synthesized by chemical esterification and transesterification. According to the reagents, the chemical method includes acid-direct esterification, acid-anhydride esterification method, acyl-chloride esterification method, transesterification method and so on.

US patent 5502045 Tatu Miettinen etc introduced a method using beta-sitostarol reacted with rapeseed oil methyl ester under vaccum at temperature 90~120°C using Na ethylate and the conversion of 98%. US Patent 6147236 provide a synthrtic route of conversion 95% that was amenable to large scale production of the esters in high yields. Oleic and stearic acid reacted with phytosterol was studied, the best parameters were 7~8 hour, 135°C, oleic or stearic acid/phytosterol mol/mol 1:1.2~1:1.3 (Chen Maobing,Huang Qin,2005).

These fatty esters sterols can be produced by chemical esterifications generally requiring higher temperature than enzymatic technologies and favoring consequently the formation of side products (e.g., dehydrated or oxysterols) and staining

2.2 Lipase-catalyzed esterification

Lipase-catalyzed esterification reactions are an important area of research in lipid chemistry, and enzyme technologies offer now a good alternative for their production allowing mild and environmental friendly reactions conditions. In the last decade, a little research has been performed on the production of phytosterol ester by lipase-catalyzed reaction. Lipase-catalyzed esterification can be acted in supercritical carbon dioxide.

2.2.1 Lipase-catalyzed esterification

Yuji shimidae etc attempted to synthesize steryl esters of PUFA by enzymatic methods (Yuji shimidae, 1999). Among lipases used, *Pseudomonas* lipase was the most effective for the synthesis of cholesteryl docosahexaenoate. When a mixture of cholesterol/docosahexaenoic acid(3:1,mol/mol), 30%water, and 3000units/g of lipase was stirred at 40°C for 24h, the esterification extent attained 89.5%. Sterols contained in three different steam distillates by physical refining by Weber etc have been converted to a high degree of long-chain acyl esters via esterification and/or transesterification with fatty acids and/or triacylglycerols using lipase from *Candida rugosa* as biocatalyst in vacuo (20-40mbar) at 40°C, 30~40°C. The lipase active could be stable used twice in vacuum but after the third time the lipase activity drop seriously (Weber N., 2001; Weber N.,2002).

Vu etc produced the sitosteryl esters from CLA and MCFAs using various lipases as a biocatalyst, Among lipases, AYS (from *C. rugosa*) was the most effective for the synthesis of sitosteryl esters in the presence of either water (maximum 26.8%) or hexane (maximum 28.3%) during a 48 h reaction, and the effect in non-polar insolvent was better than in polar condition. The optimized parameters were 55°C, magnetic whisking rate 175rpm, mol/mol of CLA with phytosterol 3:1, 48h (Vu, 2004).P.Villeneuve etc established the feasibility of lipase-catalyzed esterification of canola phytosterols with oleic acid. Among the tested lipases, namely plant lipases from *Candida rugosa* to be the most. When optimal enzyme load (5%) or temperature (35°C) were determined to allow a final production of steryl esters close to 85% after 72 h.

Condensation condition was studied for lipase-catalyzed synthesis of β- sitosterol ester with conjugated linoleic acid (CLA) in organic solvent (Li Ru, 2006).N-hexane as the reaction solvent, 4A molecular sieve as desiccant. The fittest parameters were the molar ratio 1:1,50 °Cfor 72 hours, the amount of molecular sieve 60 mg/ mL. The conversion of β-sitosterol ester of CLA was 72. 63 %.

As the difference of the reagents, lipase kinds and origin, there are differente yield of esterification from 25%~96%. While the lipase from *C. rugosa* effects better, and as the amount of lipase is enhanced or the mol/mol of fatty acid between phytosterol enlarged or in a polar condition, the rate of esterification will be increscent.

2.2.2 Lipase-catalyzed esterification in supercritical carbon dioxide

Jerry W. King et al evaluated several enzymes to determine the best catalyst and optimal conditions for the reaction between various fatty acids and cholesterol or sitostanol in SC-CO_2. The lipase derived from *Burkholderia cepacia*, Chirazyme L-1 was determined to be the most selective for facilitating the desired reactions. Fatty acids C_8–C_{18}, pressures between 20.7 MPa and 31 MPa, a temperature range of 40–60 °C, along with variable flow rates, and initial static hold times were used to evaluated the feasibility of the above reaction. The yield of the cholesterol esters, as measured by supercritical fluid chromatography (SFC), ranged from 90% for caprylic acid to 99% for palmitic acid, while the corresponding reaction between sitostanol and the same fatty acids produced yields of 92% for caprylic acid and 99% for palmitic acid, respectively.

3 Conclusion

Blood cholesterol levels in human beings in the present era still reflects efficacy and safety [36]. The safety of phytosterols has been affirmed by government agencies such as the US Food and Drug Administration and the European Union Scientific Committee. Scholarly reviews [36,37,108-110] have all confirmed the health benefits and safety of phytosterols. The use of phytosterol esters are inhibitted by solution.while the solution could be increased by esterification found, phytosterols are widely used.

References

Pollak OJ (1953). Reduction of blood cholesterol in man. Circulation 7,702–6.

Beveridge JMR, Haus HL, Connel WL (1964). Magnitude of the hypocholesterolemic effect of dietary sistosterol in man. J Nutr83,119–22.

Lees AM, Mok HY, Lees RS, et al(1977). Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol

balance. Atherosclerosis 28,325
Jones P J, Ntanios F Y (2000). Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytosterol esters. J. Lipid Res.41, 697—705.
Pollak OJ, Kritchevsky D. Sitosterol (1981). Basel7 S. Karger.
Ostlund Jr RE, McGill JB, Zeng C-M, et al(2002). Gastrointestinal absorption and plasma kinetics of soy d-5-phytosterols and phytostanols in humans. Am J Physiol Endocrinol Metab282,911-916.
Salo P, Wester I, Marangoni A (2002). Phytosterols In: Gunstone F, editor. Lipids for functional foods and nutraceuticals. Dundee, Scotland: The Oily Press Inc, 183–224.
Shipley RE, Pfieffer RR, Marsh MM, et al (1958). Sitosterol feeding: chronic animal and clinical toxicity and tissue analysis. Circ Res 6,373- 82.
Baker VA, Hepburn PA, Kennedy SJ, et al (1999). Safety evaluation of sitosterol esters. Part 1. Assessment of oestrogenicity using a combination of in vivo and in vitro assays. Food Chem Toxicol37, 13- 22.
Hepburn PA, Horner SA, Smith M(1999). Safety evaluation of phytosterol esters. Part 2. Subchronic 90day oral toxicity study on phytosterol esters-a novel functional food. Food Chem Toxicol37, 521- 32.
Ayesh R, Weststrate JA, Drewitt PN, et al (1999). Safety evaluation of phytosterol esters. Part 5. Faecal shortchain fatty acid and microflora content, faecal bacterial enzyme activity and serum female sex hormones in healthy normolipaedemic volunteers consuming a controlled diet either with or without a phytosterol ester–enriched margarine. Food Chem Toxicol37,1127- 38.
Sanders DJ, Minter HJ, Hower D, et al (2000). The safety evaluation of phytosterol esterol. Part 6. The comparative absorption and tissue distribution of phytosterols in the rat. Food Chem Toxicol,485- 91.
Wolfreys AM, Hepburn PA (2002). Safety evaluation of phytosterol esters. Part 7. Assessment of mutagenic activity of phytosterols, phytosterol esters and the cholesterol derivative, 4-cholesten-3-one. Food Chem Toxicol40,461- 70.
Lea LJ, Hepburn PA, Wolfreys AM, et al (2004). Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. Food Chem Toxicol42,771- 83.
Malini T, Vanithakumari G (1990). Rat toxicity studies with b sitosterol. J Ethnopharmacol28,221.
Pol.Industrial cami Rál (2006). Request for scientific evaluation of "Substantial equivalence" for Lipofoods'phytosterol product, intended to be used in specified foods and under regulation EC 258/97 of the European Parliament.Lipofoods, 1-28.
Tatu Miettinen,Hannu V.(1996).Use of a stanol fatty acid esteer for reducing serum cholesterol level.
John D.Higgins, Ⅲ, Ft.Washington, Pa.(2000).Prepation of sterol and stanolesters.
Chen Maobin,Huang Qin (2005).Synthesis of phytosterois stearate. Journal of Hubei University of Technology, 20(1), 1-3.
Shimada Y., Yoshinori Hiroba, Takashi Baba, et al(1999),. Enzymatic synthesis of steryl esters of polyunsaturated fatty acids. J.A.O.C.S, 76(6):713-716.
Weber N (2001). Fatty acid Steryl, stanyl, and steroids esters by Esterification and Transesterification in vacuo using Candida rugosa lipase as Catalyst. J. Agric. Food. Chem., 49, 67-71.
Weber N(2002). Cholesterol-lowering food additives: Lipase-catalyzed preparation of phytostyerol and phytostanol esters. Food Research International, 35,177-181.
Vu P.L. Shin J. A., Lim C. H., et al (2004). Lipase-catalyzed production of phytosterol esters and their crystallization behavior in corn oil. Food Research International, 7, 175- 180.
P. Villeneuve, F. Turon, Y. Caro, et al (2005). Lipase-catalyzed synthesis pf canola phytosterols oleate esters as cholesterol lowering agents. Enzyme and Microbial Technology, 37,150-155.
LI Rui, ZHANG Xiao-ming (2006). Lipase2catalyzed synthesis of β- sitosterol ester with conjugated linoleic acid in organic solvent.China Oils and Fats, 31(2), 56-59.
King J. W., Snyder J. M., Frykman H., et al. Sterol ester production using lipase-catalyzed reaction in supercritical carbon dioxide. Eur. Food Res.Technol, 2001, 212: 566-569.

Table 1 Studies on Cholesterol-lowering effects of phytosterol

Phytosterol or Food type	Number of subject duration	Diary dose		Reference
Soybean sterols	chicks		First showedsterol could inhibit the elevations of plasma and liver cholesterol and reduce severity of therosclerotic lesions.	Peterson et al 1951
	52 human being for 2 weeks	an average of 8.1 g of plant sterols daily (5.7-10.0 g)	an average of 17 subjects fed 13±1 g of phytosterol daily for 3 to 5 weeks showed a 20% decrease in blood cholesterol levels	Pollak et al 1953
soy sterol–supplemented butter		5.7 g of phytosterols per day	11% reduction in plasma cholesterol	Peterson et al 1956
free or sterifed sitosterol	rats		hypolipidemic	Duncan and Best et al 1956
sitosterol	human being	from 3 to 53 g/d	successfully for lowering plasma cholesterol levels and shown to be safe for half a century.	Pollak et al 1981
			Interesterify sitostanol with rapeseed oil and incorporate the formed sitostanol esters into a spread which is lower serum cholesterol in subjects with hypercholesterolemia	Miettinen et al 1995
Phytosterol in corn oil or in olive oil			-compared effects on cholesterol metabolism of phytosterol-rich corn oil and phytosterol-poor olive oil. -Phytosterol of Corn oil was more hypocholesterolemic, olive oil increased cholesterol fractional synthesis rates.	Howell et al 1998
Spreads containing stanol esters			stanol esters shown to exert equivalent cholesterol-lowering effects	Plat J, et al allikainen MA et al 2000
phytosterol-free corn oil		150or300mg added per meal	reduced cholesterol absorption by 12% or 28% respectively	Ostlund et al 2002

Phytosterol or Food type	Number of subject duration	Diary dose		Reference
spreads enriched with plant sterols	42 healthy subjects 8 weeks	Sterol content of the enriched spread 8.3%. Intake of 25 g/day	Serum total and LDL-cholesterol concentrations lowered by 7% and 10%, respectively, with the plant sterol-enriched compared to the control spread. Serum HDL-cholesterol concentration did not significantly differ between the two spreads. Apolipoprotein B concentrations lowered by 8% with the plant sterolenriched spread.	Temme et al.,2002
various enriched sitosterol food products	human being		found that the optimal daily dosage of sterols or stanols is 2 g/d,which can result in a 10% reduction in LDL-C, whereas higher doses provide only a small additional effect.	Katan et al 2003
500mL Sterol enriched Milk blend	71Healthy subjects 4 weeks for each product	- Place 0,1.2,1.6 g/day;	*Double blind, cross over placebo controlled study.* Substantial reduction of LDL cholesterol and the two treated group: no significative difference between the two administered doses.	Thomsen et al., 2004
300ml/d sterol enriched milk	39 Healthy subjects 12 weeks	2.0g/day terol ester alone or combined with 25 g/day of placebo or spread.	*single blind crossover design with 4 phases of 3-week interventions* Sterol enriched milk and sterol enriched spread were equally efficacious in lowering total and LDL-cholesterol as compared to placebo by 6-8% and 8-10%, respectively.	Noakes et al 2004
low-fat milk-based beverage	26 Healthy subjects 1 week for each product	Placebo, 2.2 g plant sterol equivalents or 2.2 plant sterol ester equivalent	*Double-blind, randomized, crossover study.*Both milks containing plant sterols and plant sterol ester reduced beta-carotene and alpha-tocopherol bioavailability and cholesterol absorption in normocholesterolemic men.	Richelle et al., 2004
4 phytosterol ester enriched low-fat foods	58 Healthy subjects 3 weeks each product	1.6 g/day of phytosterols as sterol esters.	Serum LDL cholesterol levels fell significantly by 6.5% with bread and 5.4% with cereal. Lipid-adjusted beta-carotene was lowered by 5-10% by sterols in bread and milk, respectively. Plant sterols in low-fat milk were almost three times more effective than in bread and cereal.	Clifton et al., 2004
Orange juice	72 mildly hypercholesterol emic subjects 8 weeks	placebo orange juice -or plant sterol fortified orange juice (2g/d)	Sterol supplemented orange juice significantly decreased total (7.2%), LDL(12.4%), and non- HDL cholesterol (7.8%) compared with baseline and compared with placebo. Apolipoprotein B levels were significantly decreased (9.5%) with sterol orange juice. There were no significant changes in HDL cholesterol or triglycerides with the sterol orange juice.	Devaraj et al., 2004
Margarine containing sterol	42 healthy subjects	30 g/day in 2 servings	The subjects consuming margarine with sterols showed a significant (11%) decrease in LDL-C (P<.001). After the consumption of margarine with sterols, the adhesion and aggregation time of blood platelets was significantly prolonged after collagen-epinephrine activation.	Kozlowska-Wojciechowska et al., 2003
Phytosterol-enriched margarines	85 subjects with type 2 diabetes ;12 weeks	2 x10g/day of spread with or without 8% esters.	After 4 weeks, total and LDL cholesterol were significantly reduced in the phytosterol group by 5.2 % and 6.8 %, HDL cholesterol was significantly increased in the phytosterol group	Lee et al., 2003
polyunsaturated spread	50 Healthy subjects total of 11 weeks	25 g of PUFA spread with or without 2g of sterols for 4 weeks, crossing over in the last 4 weeks to the alternate spread.	Replacing butter with a standard polyunsaturated fat spread reduced mean plasma total cholesterol concentrations by 4.6% and low-density lipoprotein cholesterol by 5.5%. Replacing butter with a polyunsaturated spread containing plant sterols reduced plasma total cholesterol by 8.9% and low density lipoprotein cholesterol by 12.3%. Plasma high density lipoprotein cholesterol concentration was the same on the three diets.	Cleghorn et al., 2003

Note :Figue comes of < Request for scientific evaluation of "Substantial equivalence" for Lipofoods' -phytosterol product, intended to be used in specified foods and under regulation EC 258/97 of the European Parliament>and< Phytosterols—health benefits and potential concerns>.

Effect of microwave drying on rapeseed's dehydrating characteristics and quality properties

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Abstract

Rapeseed is one of five key oil plants in the world, and main material of edible vegetable oil. But during its harvest and storage, the serious loss was incurred because of acidification and mildew. How to dry rapeseed speedily, effectively and safely has been a key technical problem in practical production. In this research, industrial microwave stove had been chosen as energy source for rapeseed drying. Based on mechanism of rapeseed microwave drying, the effect of microwave drying on rapeseed's dehydrating characteristics and quality properties had been investigated. By a series of experiments, analysis and measurement, it had been proved that: (1) rapeseed of initial moisture content 15%~30% could be dried up to the security storage moisture, by varying ratio of quality-power G, temperature T, heating time t and loading cycle Dc, correspondingly, meanwhile its seeded value had yet been guaranteed; field test showed that quality of its progeny had maintained the same, and its production had risen slightly. (2) microwave drying could activate the rapeseed, and enhance its germinating ability; (3) compared with other drying methods (such as hot-air and far-infrared drying), microwave drying has three stages of acceleration, constant-velocity and deceleration, the constant-velocity phase was the most longest. And drying rapeseed by microwave was innovative, economic and low energy-consumption; (4) microwave, as a radiation energy, could reduce the content of mustard-acid in "821" rapeseed from 42.7% to 22.4%, and glucosinolate from 66.09umol/g to 18.44umol/g. Not only the quality properties of rapeseed had been improved, its comprehensive utilization value had been also enhanced.

Key words: Microwave drying, Rapeseeds, Quality properties, Dehydrating characteristics

1. Introduction

Rapeseed is one of five key oil plants in the world, and is rapidly increasing crops of main material of edible vegetable oil. In general, the initial moisture content of rapeseed is 15%~30% while being harvested, but its safe storage moisture content must be below 9%. The serious loss was incurred by acidification and mildew, because it could not be dried in time after harvest. In practice, rapeseed drying has both natural and artificial drying methods. However, traditional natural drying (wind drying and drying in sun's rays) maybe be affected by natural conditions such as rainy season, and hot air drying have some disadvantages of drying unconformity and difficulty to precisely control moisture content etc. How to dry rapeseed speedily, effectively and safely has been a key technical problem in practical production. Compared with a variety of drying technology, as a new energy technology, microwave drying has unique drying mechanism with both magnetic and electrical characteristics. In this research, microwave drying technology was applied to dry rapeseed by experiments.

2. Materials and methods

2.1 Experimental materials

Black seed "821"-rapeseed, offered by Rape Breeding Center of Huazhong Agricultural University.

2.2 Experimental installations

(1) microwave drying device: consists of RE-630 microwave stove, temperature-control system, supporting system, and holders etc; (2) Drying apparatus: electronic breezing instrument EY3-2A, 101-Ⅲ electro-thermal blasting drying case with controlled temperature range from 50°C~200°C; (3) Else:MP120-1 electronic scale, culture utensil, filter paper, tweezers, etc.

2.3 Determination methods

Water determination with 105°C8h once-through oven-drying method, germination test according to GB5520-85 method, seed's oil content determination according to GB2906-82 method, fatty acid component determination according to GB10219-88 method, glucosinolate content determination with Palladium chloride method.

3. Results and discussion

3.1 Effect on seed's germination percentage

3.1.1 Design and analysis of experimental factors

Through various experimental factors matching, the rapeseed's moisture content was dried by microwave to the requirement of safe storage (under 9%), then the experiment of effect on germination percentage was carried out, the experimental factor levels were showed in Table1.

Table1 Experimental Factor Level Table

levels	ratio of quality-power /G/W/g	initial moisture content /W/%	temperature /T/°C	load cycle /Dc/min	time /t/min
upper level (+1)	0.35	30.0	65	15	90
zero level (0)	0.30	22.5	60	10	70
lower level (-1)	0.25	15.0	55	5	50
varying range	0.05	7.5	5	5	20

The experiment indicated that the factor order was initial moisture W, ratio of quality-power G, time t, interactive term of initial moisture content and ratio of quality-power W × G, temperature T, interactive term of initial moisture content and time W × t and load cycle Dc. The regression equation drawn from the experiment showed the function formula of germination percentage and other factors as:

$$F = 102.66 + 1.283W + 40G + 0.1t - 0.275T - 0.125D_C - 4.33W \times G - 0.0083W \times t$$

The notability test indicated that the regression formula was notable. Based on the result, with gradient method of once-through regression orthogonal design, the optimal area was obtained: initial moisture content W=11.25%~26.55%, ratio of quality-power G=0.23W/g~0.33W/g, temperature T=53.69°C~63.69°C, time t=47.34min~87.34min, load cycle Dc=4.54min~14.54min.

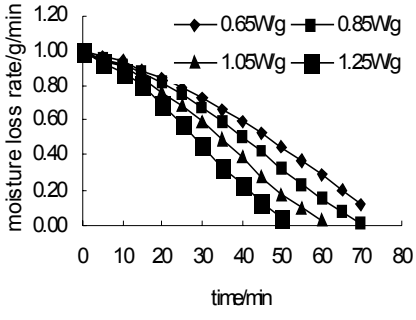

Figure 1.Result of Time and Dehydration Speed at Varying Ratio of Quality-Power

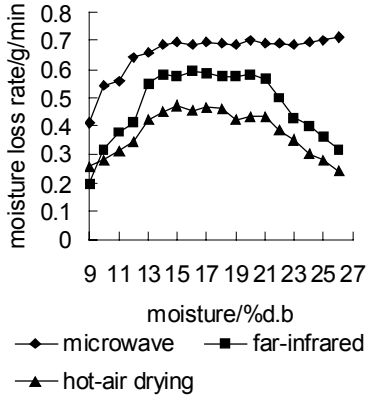

Figure 2. Result of Moisture and Dehydrating Speed at Varying Drying Methods

3.1.2 Experiment of germination potential and germination percentage

According the optimal area of experimental factors drawn from orthogonal experiment design, drying experiment was carried out under condition of 0.25W/g, 26% and 60°C, and germination experiment was carried out immediately after the dying experiment. The experiments showed that the average germination potential of rapeseed processed by microwave was 96.5%, up 4.25 percentage point than 92.25% of without microwave processing. And the germination experiment showed the seedling height in the fifth day was higher than of without microwave processing. During the microwave drying, the seed energy was enhanced because of a suitable microwave radiation dose's incentive acceleration to seed energy. Meanwhile the germination percentage of microwave processed seeds was 95.5%, almost the same as of without microwave processing 95.75%, in accord with the germination percentage demand of national standard.

3.2 Experiment and analysis of dehydration characteristics

Water and vapor diffusion exists in materials during microwave drying. The following data transformation was taken before experiment: moisture content W was transformed to water percentage WR through formula WR = [W-We] / [W_0-W_e], in it W is seed moisture content, W_0 is seed initial moisture content, W_e=11.45rh$^{0.715}$, rh is relative humidity. Figure 1 is the curve of varied ratio of quality-power and water dehydrating percentage to time, it showed that in the initial stage of drying the drying speed increased along with the ratio of quality-power, on the other hand, in the later stage, the drying speed tended to be unvaried, and every curve demonstrated that, with constant initial water content, microwave drying rapeseed was a limited diminution process.

Considering the feasibility of practical production, further comparing with far-infrared and hot-air drying, the dehydration characteristics of different drying method were obtained and shown in Figure 2. It may be drawn from the curves that in the constant speed stage the seed moisture percentage distribution was different correspondingly to the three drying methods. In microwave drying, most dehydration occurred in the constant speed stage, indicating that microwave drying is of the strongest drying ability, far-infrared drying the second, hot-air drying the weakest. The power calculation indicated that to dry seeds of the same quality, hot-air drying consumed the most power, microwave drying consumed the least power, infrared drying between them.

3.3 Effect on seed qualities

Table 2 Result of Effect of Microwave Drying on Rapeseed Quality

	repeat times	concentrated fat	fat acid							glucosinolate
			palmic acid	stearic acid	oleic acid	linolic acid	linolenic acid	diluted peanut acid	erucic acid	
natural drying	1	41.75	3.00	1.02	20.98	12.15	7.46	12.12	42.84	64.2792
	2	41.67	3.10	1.15	21.08	12.34	7.76	11.92	42.56	67.7980
	average	41.71	3.05	1.08	21.03	12.48	7.72	12.02	42.72	66.0986
microwave drying	1	42.66	4.27	1.90	40.07	18.08	7.24	7.03	22.41	18.9025
	2	42.56	4.27	1.97	39.99	18.00	7.27	6.99	22.44	17.9792
	average	42.61	4.26	1.94	40.03	18.10	7.26	7.01	22.42	18.4422

Table 3 Rapeseed Offspring Properties in Both Processing Ways

processing manners	plant height/cm	ramifying position/cm	effective ramifications	seed of single plant /num.	thousand-grain weight/g	output/kg	erucic acid /%	glucosinolate /umol/g
microwave drying	174.6	602	8.4	306.9	3.15	126.06	39.46	69.913
natural drying	169.1	55.6	7.6	279.9	3.09	118.6	40.24	78.64

Under condition of ratio of quality-power 0.25W/g, initial moisture content 26%, temperature 60°C and load cycle 5min, microwave drying and natural drying were carried out simultaneously, though determination and analysis, the effect on rapeseed qualities was obtained showed as Table 2 and Table 3. It may be drawn from the effect of microwave drying on rapeseed characteristics that rapeseed qualities had been improved by microwave processing under proper conditions, among the improved qualities, the content of erucic acid and glucosinolate reduced, oleic acid content increased, the nutritional value was enhanced, all of the above indicated that advantageous variation had occurred. The offspring of microwave processed rapeseed showed superior properties than of natural drying. It is because that microwave is a kind of electro-magnetic wave, has both effects of magnetic field and electricity field. Microwave processing materials is a physical process without causing rapeseed gene mutation. But microwave has physiological effect on rapeseed, the degree of effect and the pros and cons depend on radiation dose and coordinated technological measure.

4. Conclusion

1. According to various rapeseed initial moisture contents (15%~30%), selecting suitable ratio of quality-power (0.25W/g), setting corresponding temperature range (50°C~60°C) and drying time, rapeseed may be dried to the requirement of safe storage (under 9%). The result demonstrated that microwave drying rapeseed is feasible.

2. Microwave drying can activate seed energy, enhance seed germination potential, the average germination potential of microwave processed rapeseed is 4.25% more than of rapeseed without microwave processing. The main characters of microwave processed rapeseed were better than of natural drying.

3. The quality of rapeseed can be improved by irradiation of a certain dose of microwave as a kind of radiation energy. A comparison of microwave drying and natural drying indicated that microwave drying can enhance rapeseed nutritional value through decreasing erucic acid content, increasing oleic acid and linonic acid content, and reducing the glucosinolate content also.

4. Compared with constant-temperature hot-air drying and far-infrared drying, microwave drying is unique for the biggest dehydrating speed in constant speed stage, strong dry ability, low power consumption and good economic when drying rapeseeds of same quality and initial moisture content.

5. Microwave drying may adapt to various technological parameters by precisely controlling the moisture content and microwave output power through using a temperature controlling system. In order to enhance the dry efficiency in practical production and operation, hot-air drying and microwave drying may be combined, and continuous production may be realized through setting the length of conveyer belt and technological process.

References

Crisp J, Woods J L. The drying properties of rapeseed. J. Agric. Engng. Res., 1994,57:89~97

Adu Banjamin, Otten Lambert. Diffusion characteristics of white beans during microwave drying. J. Agric. Engng. Res., 1994,64:61~70

Wu Moucheng,1987. Common analysis methods of plant biology. Wuhan: Huazhong Agriculture University Publishing Company

Liu Houli,1990. Research trend of production and breed for rapeseed in the world. Liu Houli Science Paper Volume. Beijing: Beijing Agriculture University Publishing Company

Guan Chunyun,1985. Analysis methods of rapeseed's quality. Changsha: Hunan Science and Technology Publishing Company

P.N. Mashwari, D.W. Stanley and F.R.Van De Voort. Microwave treatment of dehulled rapeseed to inactivate myrosinase and its effect on oil and meal quality. JAOCS,1980,7:194~198

V. Shivhare, G. Raghavan. Microwave drying of corn. Transaction of ASAE,1992,35(3):947~967.

Effect of enzymatic treatment on rapeseed oil degumming and its quality

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Abstract

An experiment of rapeseed oil degumming by a phospholipase A_1 (*Lecitase® Ultra*) had been carried out for the study of effect of enzymatic treatment on the rapeseed oil degumming and its quality. The experimental results show that the *Lecitase® Ultra* plays an important role in the degumming of rapeseed oil. By doing orthogonal experiment, the optimum conditions of rapeseed oil degumming by a phospholipase A_1 were obtained as follows: pH 4.82, dosage of *Lecitase® Ultra* 150LU/kg, temperature 37°C and time of enzymatic treatment 3h. Under these conditions, the phosphorus content in obtained degummed rapeseed oil was 6.97mg/kg. At the same time, the acid value and peroxide value of the degummed oil increased, and its color had a beneficial change. In the degummed rapeseed oil, the contents of palmitic acid and oleic acid decreased, but the contents of linolenic acid, linoleic acid, eicosenoic acid and erucic acid increased, and the content of other fatty acids didn't evidently change.

Key words: Phospholipase, Rapeseed Oil,Degumming, Quality

Introduction

Enzymatic oil-degumming is a suitable process for physical refining, in which the non-hydrateable phosphatide is converted, by using a kind of phospholipase hydrolyses the ester bonds of the phospholipids in the crude oil, into a water-soluble lyso-phosphatide and is separated as a heavy phase (Klaus Dahlke, 1998). Until now, only two commercial phospholipases, which are phospholipase A2 from porcine pancreas and a phospholipase A1 from Fusarium oxysporum, are in practice for oil-degumming (Bruno H. Winter, et al., 1998; Kim Clausen, 2001). In Fig. 1, phospholipid hydrolyzing by phospholipase is shown, the non-hydrateable phosphatides consist to a high extent of phosphatidic acid, i.e., the remaining X at the phosphate group is simply a hydrogen. The lyso-phosphatide, produced in enzymatic degumming reaction, exhibits a hydrophilic group, consisting of the phosphate group and the hydroxyl group at the 1 or 2-position.

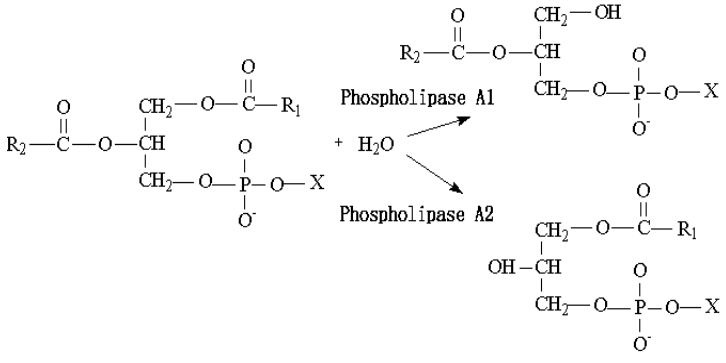

Fig.1 Phospholipid hydrolyzing by phospholipase

A generalized depiction of a phospholipid, where X = H, choline, ethanolamine, inositol, etc.

Enzymatic oil-degumming was firstly developed in the 1990s with the initial industrial plant trials by the German Lurgi company (Klaus Dahlke, 1998). This new oil-degumming process was called the "EnzyMax process", in which the phospholipase A_2 from porcine pancreas was used to change nonhydratable phospholipids into a hydratable form. Comparing with traditional degumming process, enzymatic degumming has many advantages. Apart from the reduction in the amount of acid, base and waste water during refining process, an enhancement in product yields and a reduction in operating costs can also be observed (Klaus Dahlke, 1998; Bruno H. Winter, et al., 1998; Kim Clausen, 2001; Roy S.K., et al., 2002; Ji-Guo Yang, et al., 2006). Because the porcine pancreatic phospholipase A_2 meet some problems in the application, such as limited source, expensive price and defect in function, "EnzyMax process" has not been popularized in large scale. Recently, it is found that the phospholipase A_1 from *Fusarium oxysporum* can be used in oil-degumming process(Roy S.K., et al., 2002). The phospholipase A_1 can be largely produced by fermentation, furthermore some new and excellent performance phospholipase can be obtained by filtrating, which makes enzymatic oil-degumming more economy and availability in practice.

Lecitase® Ultra is a new microbial lipase developed by Novozymes, and it is a protein-engineered carboxylic ester hydrolase from *Thermomyces lanuginosus/ Fusarium oxysporum* produced by submerged fermentation of a genetically

modified *Aspergillus oryzae*. The aim of this work was to study the oil-degumming process using *Lecitase® Ultra*, and evaluate the effect of enzymatic treatment on the rapeseed oil quality.

2 Materials and Methods

2.1 Materials

Water-degummed rapeseed oils were supplied by Zhongpai Grain & Oil Ltd (Wuhan, China), and the phosphorus content typically was 177.62mg/kg.

Phospholipase A_1 (Lecitase® Ultra, E.C 3.1.1.3) was kindly donated by Novozymes A/S, Bagsvaerd, Denmark.

Sodium hydroxide and *citric acid* were obtained from Tianda (Tianjin, China). They were of analytical grade and used without further purification.

2.2 Methods

2.2.1 Enzymatic degumming lab trial

Water-degummed rapeseed oil (100 g, 177.62mg/kg phosphate) was placed into a 250 mL conical flask fitted with stopper. The oil was heated to about 80 °C in a water bath, and then 0.16 mL of 45 % citric acid was added and homogenized for 1.5 min at 8000 rpm. After acidic reaction for 20min at 80°C under 300 rpm, the temperature of the oil was decreased to temperature preset, and a suitable amount of 4 % NaOH were added with shear mixing at 300 rpm for 5 min. After reaction completion, enzyme and water were added with shear mixing. The flask was placed in a water bath of preset temperature to begin enzymatic degumming reaction with shear mixing at 500 rpm. After certain long time incubation, oil emulsion was heated in 80°C for 10 min and then centrifuged at 4800 rpm for 20 min. After phase separation, top layer oils were collected in an airtight container for phosphorus analysis.

2.2.2 Phosphorus content analysis

Phosphorus analysis was carried out as follows: 100 mg of MgO were weighed in a porcelain dish and heated on a gas burner. A mass of 0.5–2 g of oil was added and ignited with a gas burner to become a black, hard mass, and then it was heated at 850 °C for 2 h until it turned into white ash. The phosphorus content of the ash was determined according to AOCS method Ca 12–55. All experiments were carried out in triplicate for the calculation of the mean value.

2.2.3 pH determination(Kim Clausen, 2001)

5 ml of water in oil emulsion were mixed with 5ml of MilliQ water. And then mixtures were separated by centrifugation at 4800rpm for 20min. After phase separation, top layer oil was pipetted off. The pH in aqueous phase was measured with pH electrode Mettler-Toledo. In order to compensate for the dilution effect, measurements were transformed to corrected pH values by formula ($pH_{corrected} = pH_{measured} - 0.38$).

3 Results and Discussion

3.1 Single-factor experiments

3.1.1 Effect of enzymatic treatment time on degumming of rapeseed oil

Some enzymatic degumming trials were done by way of method 2.2.1 mentioned, and the reaction conditions were controlled as follow: pH 4.82, dosage of *Lecitase® Ultra* 300LU/kg, temperature 48°C, 0.55ml of 4% NaOH, total water content 2.5% and time of enzymatic treatment 0, 1, 2, 3, 4, 5h, respectively. After degumming process was completed, the phosphorus content in the examples of degummed oil was analyzed respectively and the determined results were displayed in Fig.2.

As shown in Fig.2, within 1h of the beginning of enzymatic degumming reaction, the phosphorus content decreased very rapidly from 177.62 mg/kg to 46.08 mg/kg. The addition of citric acid buffer and *Lecitase® Ultra* had already caused together coagulation and precipitation of part of phosphatides. Therefore, the phosphorus content in the oil was substantially lower than the phosphorus content of Water-degummed oil after the acid treatment. After enzymatic degumming reaction went on for 1h, *Lecitase® Ultra* became a main factor for removal of nonhydratable phospholipids. As the result, the speed of phosphorus content decreasing became slower. Beyond 3h of the enzymatic degumming reaction, addition of reaction time could almost not reduce phosphorus content of oil. Thought of saving production time and increasing degumming efficiency, 3h was an appropriate time for this enzymatic degumming reaction.

3.1.2 Effect of pH on degumming of rapeseed oil

The degumming tests were done by way of method mentioned above, and the reaction conditions were controlled as follow: dosage of *Lecitase® Ultra* 150LU/kg, temperature 48°C, total water content 2.5% and time of enzymatic treatment 3h, and pH 4.13, 4.42, 4.65, 4.82, 4.97, 5.15, 5.39, respectively. After degumming process was completed, the phosphorus content in the examples of degummed oil was analyzed respectively and the results were shown in Fig.3.

The pH optimum of this enzyme for degumming of rapeseed oil was displayed in Fig.3. *Lecitase® Ultra* is an acidic lipase, which pH has a remarkable effect on its catalytic activity. As shown in Fig.3, *Lecitase Ultra* exhibits maximal degumming performance at pH 4.82.

3.1.3 Effect of dosage of *Lecitase® Ultra* on degumming of rapeseed oil

The enzymatic degumming reaction conditions were controlled as follow: temperature 48°C, 0.6ml of 4% NaOH, total water content 2.5% and time of enzymatic treatment 3h, and dosage of *Lecitase® Ultra* 50, 100, 150, 225, 300, 375, 450LU/kg respectively. The phosphorus content in the examples of degummed oil was analyzed respectively and the results were

displayed in Fig.4.

As shown in Fig.4, within 150LU/kg of enzymatic dosage, dosage of enzyme played an important role in effect of degumming. Beyond 150LU/kg of enzymatic dosage, addition of enzymatic dosage reduced hardly phosphorus content in the example of degummed oil. Thus, thought about economy of production, 150LU/kg was an appropriate of enzymatic dosage for rapeseed oil degumming.

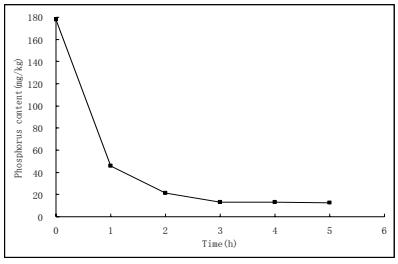

Fig.2 Effect of enzymatic treatment time on phosphorus content of degummed rapeseed oil

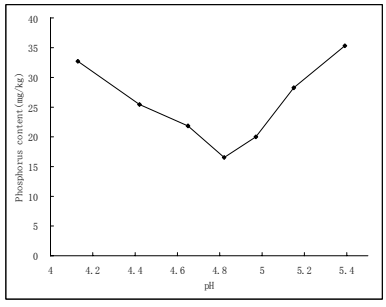

Fig.3 Effect of pH on phosphorus content of degummed rapeseed oil

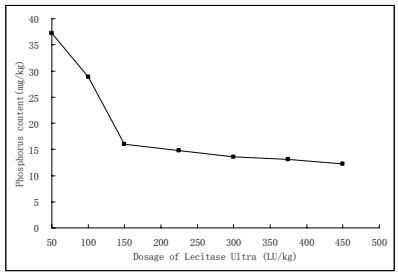

Fig.4 Effect of dosage of *Lecitase® Ultra* on phosphorus content of degummed rapeseed oil

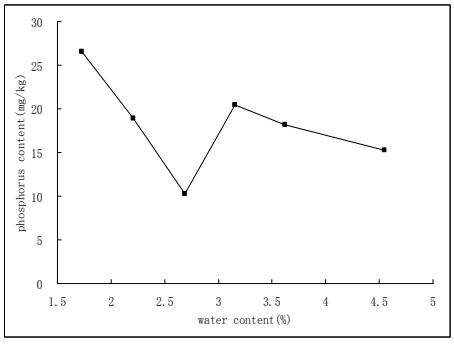

Fig.5 Effect of content of water on phosphorus content of degummed rapeseed oil

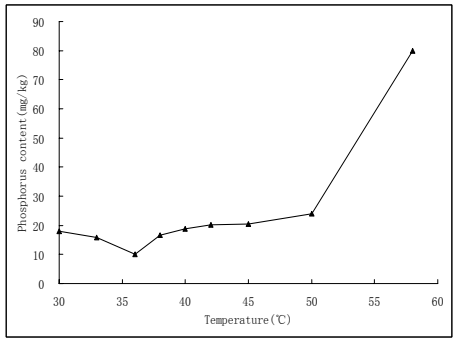

Fig.6 Effect of temperature on phosphorus content of degummed rapeseed oil

3.1.4 Effect of content of water on degumming of rapeseed oil

The enzymatic degumming reaction conditions were controlled as follow: dosage of *Lecitase Ultra* 150LU/kg, temperature 48°C, 0.6ml of 4% NaOH, time of enzymatic treatment 3h, and total water content 1.73%, 2.21%, 2.69%, 3.16%, 3.62%, 4.54%, respectively. After enzymatic degumming process was finished, the phosphorus content in the example of degummed oil was analyzed respectively and the determined results were displayed in Fig.5.

As shown in Fig.5, when total water content varied from 1.73 to 2.69% in the oil-degumming experiments, the more water was added, the better the enzymatic degumming effect was. However, when the total water content was between 2.69% and 3.16%, the phosphorus content of degummed rapeseed oil increased correspondingly. Within 3.16%~4.54% of total water content, the phosphorus content of degummed rapeseed oil reduced slowly. *Lecitase® Ultra* has ability to perform oil-degumming at reduced water content as shown in Fig. 5. At total water content 2.69%, it is still possible to degum to a final phosphorus level at 10 ppm within 3h. Therefore, the optimum water content was 2.69%.

3.1.5 Effect of temperature on degumming of rapeseed oil

The enzymatic degumming reaction conditions were controlled as follow: 0.6ml of 4% NaOH, total water content 2.69%, time of enzymatic treatment 3h, and dosage of *Lecitase® Ultra* 150LU/kg, and temperature 30, 33, 36, 38, 40, 42, 45, 50, 58°C, respectively. The phosphorus content in the example of degummed oil was analyzed respectively and the results were displayed in Fig.6.

Temperature is an important effect factor for enzymatic catalytic reaction. As shown in Fig.6, *Lecitase® Ultra* had a better catalytic activity at normal temperature. When reaction temperature varied between 30°C and 45°C, the phosphorus content of degummed rapeseed oil changed little, and *Lecitase® Ultra* had a best effect of degumming for rapeseed at 38°C. When reaction temperature was increased over 50°C, *Lecitase® Ultra* catalytic activity reduced quickly and phosphorus content of degummed rapeseed oil increased rapidly.

3.2 Optimized experiment

Referenced results of single factor experiments, pH, dosage of *Lecitase® Ultra* and temperature were chosen as important factors and using $L_9(3)^4$ model designed an orthogonal experiment for optimizing enzymatic degumming process. Factors and levels in the experiment of degumming by enzyme were shown in table 1.

With phosphorus content of degummed rapeseed oil as evaluation index, total water content 2.69% and enzymatic reaction time 3h, orthogonal experiments were done, and then experiments results were shown in table 1.

Table 1 Result of orthogonal experiment

Test number	pH	Dosage of *Lecitase Ultra* LU/kg	Temperature °C	Empty List	Phosphorus content mg/kg
1	4.65	150	33	1	15.74
2	4.65	225	37	2	12.39
3	4.65	300	41	3	14.26
4	4.82	150	37	3	6.97
5	4.82	225	41	1	9.26
6	4.82	300	33	2	8.03
7	5.06	150	41	2	18.22
8	5.06	225	33	3	13.35
9	5.06	300	37	1	11.59
Average 1	14.13	13.64	12.37	12.20	
Average 2	8.09	11.66	10.32	12.88	
Average 3	14.38	11.29	13.91	11.53	
R	6.29	2.35	3.59	1.35	

From results of range analysis showed in table 1, it was seen that sequence, three factors effect on enzymatic degumming, as follow: pH>temperature> dosage of *Lecitase® Ultra.* As shown in table 2, a conclusion could be made that pH have a remarkable effect on degumming rapeseed oil, but effect of temperature and dosage of *Lecitase® Ultra* on degumming rapeseed oil were inconspicuous. Through analysis of range for the results of orthogonal experiment, the optimum conditions of rapeseed oil degumming by *Lecitase® Ultra* were obtained as follows: pH 4.82, dosage of *Lecitase® Ultra* 300LU/kg, temperature 37°C and time of enzymatic treatment 3h. On these conditions, the phosphorus content in obtained degummed rapeseed oil was 6.32mg/kg. Contrast with No. 4 of table 1, phosphorus content induced 0.65 mg/kg, but dosage of *Lecitase® Ultra* doubled. Thus, thought of products cost, The reaction conditions of No. 4 were determined as optimum parameter of rapeseed oil degumming by *Lecitase® Ultra*.

Table 2 Analysis of variance for the result of orthogonal experiment

Variation source	Quadratic sum of deviation	Degree of freedom	F-ratio	$F_{\alpha=0.05}$	Significance
pH	76.294	2	27.967	19	*
Dosage of *Lecitase Ultra* LU/kg	9.599	2	3.519	19	
Temperature°C	19.507	2	7.151	19	
Empty List	2.728	2	1	19	
Error	2.73	2			

3.3 Effect of enzymatic treatment on quality of the rapeseed oil

3.3.1 Effect on acid value of the oil

Acid value of nine kinds of oils examples, which chosen from rapeseed oil degummed by enzyme at random, were determined and contrast with water-degummed rapeseed oil (Fig.7). As shown in Fig. 7, after enzymatic degumming process, acid value of degummed oil had a certain degree rise and the addition range was between 0.28 mgKOH/g and 1.04 mgKOH/g, most acid value of degummed oil were under 3.00mgKOH/g.

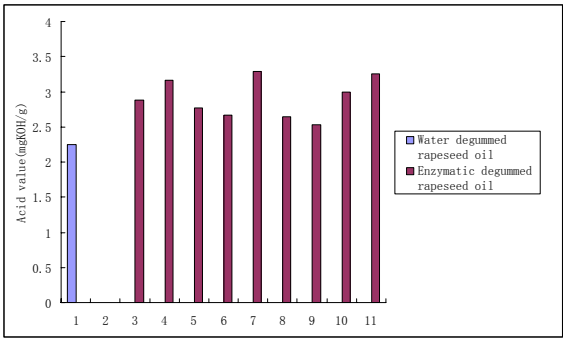

Fig.7 Effect of enzymatic treatment degumming on acid value of rapeseed oil

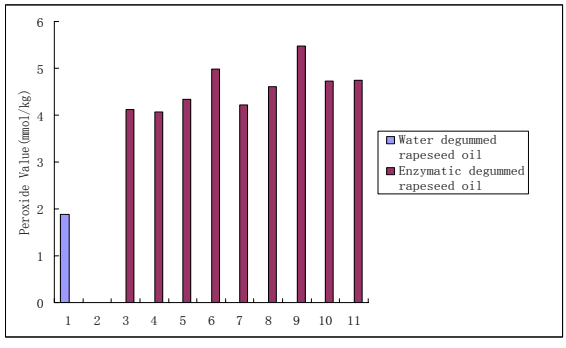

Fig.8 Effect of enzymatic treatment degumming on peroxide value of rapeseed oil

It is well known that one fatty acid will be removed from one phospholipid molecule in the enzymatic degumming process. As a result, there will raise content of the free fatty acids (FFA). In this study, it was found that the amount of FFA increase fitted well to that of the decrease of phosphorus. Therefore, it was concluded that the increase of free fatty acids was the consequence of the hydrolysis of phospholipids rather than triglycerides. It was an interesting phenomenon that the *Lecitase® Ultra* enzyme was able to identify only the phospholipids as substrate, and did not hydrolyze the triglyceride in the

enzymatic oil degumming system, although the lipase activity of the *Lecitase® Ultra* enzyme was remarkable in the other system (Ji-Guo Yang, et al., 2006). The mechanism of this enzymatic reaction needs to be explored in the future research.

3.3.2 Effect on Peroxide Value of the oil

Peroxide value of nine kinds of oils examples, which chosen from rapeseed oil degummed by enzyme at random, were determined and contrast with water-degummed rapeseed oil (Fig.8). As shown in Fig. 8, after enzymatic degumming process, peroxide value of degummed oil had a certain degree rise, and most scales reach 3.60 mmol/kg. However, all of peroxide value of nine kinds of oils examples below 6.00 mmol/kg, most were between 4.00 and 5.00 mmol/kg.

3.3.3 Effect on fatty acids composition of the oil

The colors of oils were determined by Lovibond Tintometer with 2.54 cm cell. As shown in table 3, the color of enzymatic degummed oil was better than water degummed oil. And a conclusion could be made that color of rapeseed oil became thinness by enzymatic degumming. The content of palmitic acid and oleic acid decreased, but the content of linolenic acid, linoleic acid, eicosenoic acid and erucic acid increased, and other the content of fatty acid didn't evidently change (table 3).

Table 3 Effect of enzymatic treatment degumming on color and fatty acids composition of rapeseed oil

Kind of oil / Test Items	Water degummed rapeseed oil	Enzymatic degummed rapeseed oil
Color (Lovibond Tintometer with 2.54 cm cell)	Y35 R4.6	Y35 R4.0
Myristic Acid %	—	0.1
Palmitic acid %	4	3.3
Palmitoleic Acid %	0.3	0.3
Heptadecanoic Acid %	0.1	0.1
Stearic acid %	1.7	1.6
Oleic acid %	38.6	33.7
Linoleic acid %	14.4	14.7
Linolenic acid %	6.5	7.1
Arachidic Acid %	0.9	0.8
Eicosenoic Acid %	7.8	8.6
Eicosadienoic Acid %	0.4	0.3
Docosanoic Acid %	0.5	0.5
Erucic Acid %	25.1	29

4 Conclusions

4.1 The optimum conditions of rapeseed oil degumming by a phospholipase A_1 (*Lecitase® Ultra*) were obtained as follows: pH 4.82, dosage of *Lecitase® Ultra* 150LU/kg, temperature 37°C and time of enzymatic treatment 3h. On these conditions, the phosphorus content in obtained degummed rapeseed oil was 6.97mg/kg. pH have a remarkable effect on degumming rapeseed oil by *Lecitase® Ultra*, but effect of temperature and dosage of *Lecitase® Ultra* on degumming rapeseed oil was inconspicuous.

4.2 After rapeseed oils were degummed by enzyme, the acid value and peroxide value of the degummed oil rose, the most increased value was 1.04mgKOH/g and 3.60 mmol/kg, respectively. And its color had a beneficial change. In degummed rapeseed oil, the content of palmitic acid and oleic acid decreased, but the content of linolenic acid, linoleic acid, eicosenoic acid and erucic acid increased, and other the content of fatty acid didn't evidently change.

References

Bruno H. Winter, Kornelia Titze, Volker Marschner(1998). Application of phospholipases in the edible oil industry. Fett/Lipid, 100:152-156.
Ji-Guo Yang, Yong-Hua Wang, Bo Yang,et. al(2006). Degumming of Vegetable Oil by a New Microbial Lipase. Food Technol. Biotechnol., 44 (1): 101–104.
Kim Clausen(2001). Enzymatic oil-degumming by a novel microbial phospholipase. Eur. J. Lipid Sci. Technol., 103:333-340.
Klaus Dahlke(1998). An Enzymatic Process for the Physical Refining of Seed Oils. Chem. Eng. Technol., 21(3):278-281.
Roy S.K., Rao B.V.S.K.,Prasad R.B.N.(2002). Enzymatic degumming of rice bran oil. J.Am.Oil Chem. Soc., 79:845-846.

Protective effects of soft ripened cheese (camembert) containing vegetable oil (rapeseed) compared to classical dairy fat cheese on the severity of atherogenic markers in hamsters fed hyperlipidemic diets

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Abstract

Milk fat is rich in saturated fatty acids (SFA) known to increase plasma cholesterol, but we previously showed that there was some benefit to cheese consumption compared to milkfat: at equal fat content, cheese was less atherogenic than milkfat*. In this study we evaluated the impact of diets including cheese prepared with different fat sources: Veg-cam cheese *"Primevere"* prepared with vegetable rapeseed oil (poor in SFA) compared to Dairy-cam cheese prepared with milkfat (camembert) rich in SFA and cholesterol.

Two groups of 9 hamsters were fed with equal fat and protein contents from cheese sources: Dairy-cam or Veg-cam. Diets were obtained by blending lyophilized whole cheese with the chow-based diet, to provide up to 10% or 20% (by weight) of fat. Animals were fed for 5wks with the 10% fat diets and then with the 20% fat diets till 17wks. The atherogenic impact was evaluated after both 10% and 20% fat diets on plasma lipids: total cholesterol (TC), LDLC, non-HDLC, atherogenic ratio non-HDLC/HDLC, and after the 20% fat diets by measuring the aorta lesions severity expressed by its cholesteryl esters (CE) content (by GLC).

Results showed that after the 10% fat diets, Veg-cam induced lower levels of plasma lipids than Dairy-cam (TC:- 29% $p<0.001$, non-HDL/HDLC:-45%$p<0.05$). Interestingly, the increase of fat intake from 10% to 20% induced opposite changes in plasma lipids: a decrease with Veg-cam (TC:-16% $p<0.001$), and an increase with Dairy-cam (TC:+20% $p<0.05$), resulting in much more prominent differences after the long term 20% fat diets (TC:- 51%$p<0.001$). However, the increase of fat intake from 10% to 20% did not modified the plasma atherogenic ratio non-HDLC/HDLC within each type of cheese diet: Veg-cam ratio (0.56vs0.56) and Dairy-cam ratio (1.06vs113). The atherosclerotic fatty streaks evaluated by the CE levels of the aortas were also dramatically reduced: four time less in Veg-cam group than in the Dairy-cam group.

At equal fat and protein content, cheese prepared with rapeseed vegetal oil is less atherogenic than the classical dairy cheese and may contribute to the recommended SFA reduction intake. **B. Delplanque, A. Thaminy, G. Agnani, JC. Martin, K. Bensharif, D Gripois. Comparison of the effects of cheese or butter on the severity of atherogenic markers in hamsters fed an hyperlipidemic diet. ISA 2006 Rome*

Key words: Rapeseed, oil, cheese, saturated, unsaturated, alphalinolenic fatty acids, plasma and aorta lipids.

Introduction

Milk fat is rich in saturated fatty acids (SFA) known to increase plasma cholesterol, but we previously showed that there was some benefit to cheese consumption compared to milkfat consumption: at equal fat content and quality, cheese was less atherogenic than milkfat. The plasma atherogenic ratio nonHDL-C/HDL-C was reduced by 42% ($p<0.01$) and was correlated to the reduction (-25% $p<0.05$) of the levels of Cholesteryl esters (CE) deposit in the hamsters aortas (**B. Delplanque, A. Thaminy, G. Agnani, JC. Martin, K. Bensharif, D Gripois. Comparison of the effects of cheese or butter on the severity of atherogenic markers in hamsters fed an hyperlipidemic diet. ISA 2006 Rome).*

In this study, the impact of hyperlipidemic diets including cheese prepared with different fat sources was evaluated on atherogenic parameters of hamsters plasma and aorta: Veg-cam cheese *"Primevere"* prepared with vegetable rapeseed oil (poor in SFA) was compared to classical Dairy-cam cheese prepared with milkfat (camembert) rich in SFA and cholesterol.

Methods

Diets **:** Two diets were carried out with cheese (camembert) sources. Veg-cam diet was obtained by blending lyophilized whole cheese prepared with rapeseed oil with the chow-based diet and the Dairy-cam was similarly prepared with the classical cheese prepared with milk fat, to provide up to 10% or 20% (by weight) of fat for both. *Experimental procedures:* Two groups of 9 hamsters were fed with Veg-cam or Dairy-cam respectively for 5 weeks with the 10% fat diets and then for 12 weeks with the 20% fat diets. At the end of these 5 and 12 week-periods, the hamsters were fasted overnight and blood was withdrawn under anaesthesia by intra-cardiac puncture (0.5mL and 3mL respectively). After the second period, aortas and other organs were removed, quickly frozen in liquid N2 and stored at -80°C until lipid analysis. Plasma lipids and lipoproteins were measured by enzymatic procedures (using commercial kits on automatic analyzer). The aorta lesions severity was evaluated by GLC as expressed by its cholesteryl ester (CE) content.

Statistical analysis were performed by student's t-test (α, $p<0.05$, γ, $p<0.001$).

Results

Lipids and lipoproteins After 5 weeks of 10% fat diets, Veg-cam induced lower levels of plasma lipids than Dairy-cam (TC:-29% $p<0.001$, non-HDL/HDLC:-45% $p<0.05$) (fig.1). The differences were more prominent after the 12 weeks of 20% fat diets (TC:-51% $p<0.001$) (fig.2).

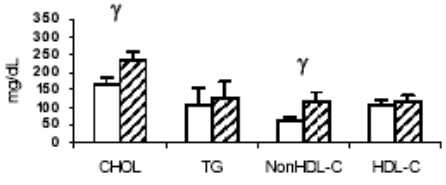

fig.1 Lipids and lipoproteins after 5 weeks of 10% fat diets

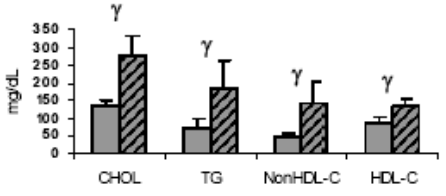

fig.2 Lipids and lipoproteins after 12 weeks of 20% fat diets

Interestingly, the increase of fat intake from 10% to 20% induced opposite changes in plasma lipids and lipoproteins: a decrease with Veg-cam (TC:-16% $p<0.01$), and an increase with Dairy-cam (TC:+20% $p<0.05$), resulting in much more prominent differences after these two long term 20% fat diets. The resulting plasma atherogenic ratio non-HDLC/HDLC was almost double with the Dairy-cam diet compared to the Veg-cam diet (1.06 vs 0.56 $p< 0.05$) (fig.3). However, the increase of fat intake from 10% to 20% did not modify this atherogenic ratio within each type of cheese diet: Veg-cam ratio (0.56 vs 0.56) and Dairy-cam ratio (1.06 vs 1.13).

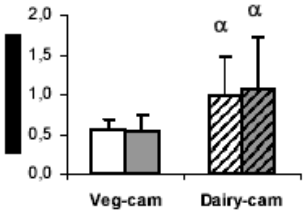

fig.3 Atherogenic ratios after 10%and 20% fat diets

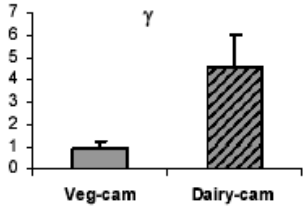

fig.4 Relative CE deposit in aorta

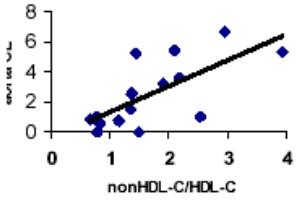

fig.5 Correlation between aorta CE and plasma nonHDL-C/HDL-C

Aorta lipids

At the end of the 20% fat diets, the aorta lesion severity (fatty streaks) evaluated by the measurement of the CE level was also dramatically reduced: four time less in Veg-cam group than in the Dairy-cam group (fig.4) and was correlated to the nonHDL-C/HDL-C ratio ($r_2 = 0{,}513$, $p<0.01$) (fig.5).

Conclusion

Within our hyperlipidemic protocole for hamsters and at equal fat and protein content, cheese prepared with rapeseed oil is less atherogenic than the classical dairy cheese and may contribute to the recommended SFA reduction intake.

Optimization of polysaccharides by acid extraction from rapeseed meal

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Abstract

The quadratic orthogonal rotation design with 5 factors was used to study the effects of acid concentration, liquid/solid ratio, time, temperature and extracting times on the yield of polysaccharide by acid extraction from rapeseed meal, the functional model of yield to 5 factors was established by RSREG software which is part of SAS8.0. The mathematical model for the simulation was presented and the regression equation is significant. The analysis on this model showed that the effect order of 5 factors on yield was: temperature > extracting times> time> liquid/solid ratio> acid concentration. The optimum technical condition was as follows: the rapeseed meal was marinated with 18-22 times 0.10-0.14mol/L HCl at 100°C for 1.4-1.8 hours, the extracting times were 4-5.The yield of soluble polysaccharide by acid extraction from rapeseed meal was over 3.1% under this condition.

Key words : polysaccharide from rapeseed meal, acid extraction, orthogonal rotation design, mathematical model, yield efficiency analysis

Introduction

Comprehensive utilization of rapeseed meal can increase economic efficiency of the rapeseed. Except for protein, polyphenols and phytic acid, polysaccharides is another major available composition of rapeseed meal. So far, there was almost no report on polysaccharides from rapeseed meal either at home or abroad, and the systemic research on it is just at initial stage. The anti-oxidative effect of polysaccharides from rapeseed meal has been approved by the early experiments (Yan Fengwei et al., 2004), which was a basis on the further research of polysaccharide of rapeseed meal.

Polysaccharides extracted from plants by acids have been testified to have apparent bio-functions which was not the same as correspondingly hot-water treatment (Dong Hongxin et al., 2004, Lin Yuman et al.,1998). Research and utilization on acidic polysaccharide of rapeseed meal was desirable to offer a valuable and abundance source of functional ingredient, and improve rapeseed comprehensive utilization efficiency. Presently there was no report on polysaccharides extracted by acid from rapeseed meal. This experiment take acid as extract solvent, quadratic rotation-orthogonal composite experimental design was employed with acid concentration, liquid/solid ratio, time, temperature and extracting times as variables to study the effects on the yield of polysaccharides. Optimization analysis was used to select the optimum conditions. This experiment aimed to improve the technical and yield of the polysaccharides extraction from rapeseed meal, and offer basic information to the next study.

Material and Methods

Experimental procedure: Dehulled double-low rapeseed cake and meal → extracted with 80% ethanol → plant residues → extracted with dilute hydrochloric acid → concentrated → deposited with 95% ethanol → dissolved deposition with water → determining yield of polysaccharides (Ruan Zheng et al., 2005, Zheng Xiaodong et al., 2000, Robert J et al., 2000)

Statistical manner and software : Quadratic rotation-orthogonal composite experimental design. RSREG software (part of SAS8.0) (Jiang Qiuyan et al., 2005, Yang Aimei et al., 1998)

Experimental arrangement: Quadratic rotation-orthogonal composite experimental design (1/2conduct) with 5 variables which is acid concentration (X_1), liquid/solid ratio (X_2), time (X_3), temperature (X_4) and extracting times (X_5). Response function is yield of polysaccharides. Every variable with 5 levels, the Factors and levels of the experiment show in Table.1. (Yang Aimei et al., 1998)

Table 1 Factors and levels of the experiment

Code	X_1(mol/L)	X_2 (g/ml)	X_3(hour)	X_4(°C)	X_5
-2	0.1	1:5	1	20	1
-1	0.2	1:10	1.5	40	2
0	0.3	1:15	2	60	3
1	0.4	1:20	2.5	80	4
2	0.5	1:25	3	100	5

Result

The effects of the five variables (acid concentration, liquid/solid ratio, time, temperature and extracting times) on the response functions (yield of polysaccharide) were shown in Table.2, The analysis of partial regression coefficient significance was shown in Table.3.

Table 2 Structured matrices and yield result

No.	X_1	X_2	X_3	X_4	X_5	Y(%)
1	1.000	1.000	1.000	1.000	1.000	1.64
2	1.000	1.000	1.000	-1.000	-1.000	0.69
3	1.000	1.000	-1.000	1.000	-1.000	1.89
4	1.000	1.000	-1.000	-1.000	1.000	0.51
5	1.000	-1.000	1.000	1.000	-1.000	1.64
6	1.000	-1.000	1.000	-1.000	1.000	0.51
7	1.000	-1.000	-1.000	1.000	1.000	2.58
8	1.000	-1.000	-1.000	-1.000	-1.000	0.46
9	-1.000	1.000	1.000	1.000	-1.000	2.14
10	-1.000	1.000	1.000	-1.000	1.000	0.63
11	-1.000	1.000	-1.000	1.000	1.000	2.17
12	-1.000	1.000	-1.000	-1.000	-1.000	0.35
13	-1.000	-1.000	1.000	1.000	1.000	2.11
14	-1.000	-1.000	1.000	-1.000	-1.000	0.51
15	-1.000	-1.000	-1.000	1.000	-1.000	1.54
16	-1.000	-1.000	-1.000	-1.000	1.000	0.45
17	2.000	0.000	0.000	0.000	0.000	1.57
18	-2.000	0.000	0.000	0.000	0.000	0.95
19	0.000	2.000	0.000	0.000	0.000	1.29
20	0.000	-2.000	0.000	0.000	0.000	0.95
21	0.000	0.000	2.000	0.000	0.000	1.10
22	0.000	0.000	-2.000	0.000	0.000	0.98
23	0.000	0.000	0.000	2.000	0.000	2.05
24	0.000	0.000	0.000	-2.000	0.000	1.09
25	0.000	0.000	0.000	0.000	2.000	1.23
26	0.000	0.000	0.000	0.000	-2.000	0.67
27	0.000	0.000	0.000	0.000	0.000	1.39
28	0.000	0.000	0.000	0.000	0.000	1.51
29	0.000	0.000	0.000	0.000	0.000	1.50
30	0.000	0.000	0.000	0.000	0.000	1.36
31	0.000	0.000	0.000	0.000	0.000	1.62
32	0.000	0.000	0.000	0.000	0.000	1.36
33	0.000	0.000	0.000	0.000	0.000	1.14
34	0.000	0.000	0.000	0.000	0.000	1.04
35	0.000	0.000	0.000	0.000	0.000	1.48
36	0.000	0.000	0.000	0.000	0.000	1.26

Regression equation for the response functions in the actual level of variables:

$$Y=1.331477+x_1*0.026250+x_2*0.063750+x_3*0.017083+x_4*0.552917+x_5*0.093750+(x_1*x_1)*(-0.007727)+x_1*x_2*(-0.055625)+x_1*x_3*(-0.075625)+x_1*x_4*(-0.066875)+x_1*x_5*(-0.055625)+(x_2*x_2)*(-0.042727)+x_2*x_3*(-0.011875)+x_2*x_4*0.021875+x_2*x_5*(-0.061875)+(x_3*x_3)*(-0.062727)+x_3*x_4*(-0.060625)+x_3*x_5*(-0.081875)+(x_4*x_4)*0.069773+x_4*x_5*0.059375+(x_5*x_5)*(-0.085227)$$

Analysis of variance: $R=0.9152$, $F=(R^2/20)/((1-R^2)/11)=2.84$, $P=0.0395$.Regression equation indicated significance, fitting is in condition.

The results indicated that among the variables, acid concentration (X_1) had a significant positive effect on the response function (yield of polysaccharides). Time (X_3), extracting times (X_5) had a positive effect on the response function. Among the various interactions, temperature (X_4) and liquid/solid ratio (X_2) had the positive effect followed by the positive effect of extracting times (X_5), all the others had the insignificant negative effects with each other. The quadratic effect of temperature had significant positive effect on the yield of polysaccharides, all the others had negative effect on the yield. It could be found that temperature take the most important role on the yield of polysaccharide apparently.

Some pretests have been done before the composite experimental design. Results of the pretest showed that when the acid concentration was 0.05~0.15mol/L, the yield of polysaccharides increased with the increase of acid concentration, nevertheless increased the acid concentration continuously, the yield of polysaccharides increased mildly and even decreased when beyond 0.3 mol/L; when the liquid/solid ratio was 1:5~1:30, yield of polysaccharides increased with the increase of liquid/solid ratio and increased mildly when it beyond 1:15; when time was 0.5~3.5 hours, yield of polysaccharides increased along with the increase of time and increased mildly when beyond 2 hours; when temperature was 20~100°C, yield of polysaccharides increased with the increase of temperature observably; when extracting times was 1~6, polysaccharides can be extracted completely from the material after 5 times extraction. The five levels of every variable selected based on above results, so despite the significance analysis indicated that except temperature, the other variables all had the insignificant effects on the response function, the optimum values of every factors had been included in its range. Similar conclusions could

be certified from the optimization analysis.

Table 3 analyze of partial regression coefficient significance

Variable Intercept	DF	Estimate	Error	T	Value
	1	1.26716	0.08382	15.12	<.0001
x1	1	0.02625	0.08025	0.33	0.7497
x2	1	0.06375	0.08025	0.79	0.4438
x3	1	0.01708	0.08025	0.21	0.8353
x4	1	0.55292	0.08025	6.89	<.0001
x5	1	0.09375	0.08025	1.17	0.2674
x11	1	-0.00773	0.07259	-0.11	0.9171
x12	1	-0.05563	0.09829	-0.57	0.5828
x13	1	-0.07563	0.09829	-0.77	0.4579
x14	1	-0.06688	0.09829	-0.68	0.5103
x15	1	-0.05562	0.09829	-0.57	0.5828
x22	1	-0.04273	0.07259	-0.59	0.5680
x23	1	-0.01187	0.09829	-0.12	0.9060
x24	1	0.02188	0.09829	0.22	0.8280
x25	1	-0.06188	0.09829	-0.63	0.5419
x33	1	-0.06273	0.07259	-0.86	0.4060
x34	1	-0.06063	0.09829	-0.62	0.5499
x35	1	-0.08187	0.09829	-0.83	0.4226
x44	1	0.06977	0.07259	0.96	0.3571
x45	1	0.05938	0.09829	0.60	0.5580
x55	1	-0.08523	0.07259	-1.17	0.2652

Discussion

Main factor effect analysis: Analysis of regression equation and regression coefficient indicated 5 variables effected on yield arranged with significance was: temperature > extracting times > time> liquid/solid ratio> acid concentration.

Individual factor effect analysis: Analysis of quadric regression model by reducing dimensionality, the effect equations of yield were:

$$Y_1 = 1.331477 + x_1 * 0.026250 + (x1 * x1) * (-0.007727)$$

$$Y_2 = 1.331477 + x_2 * 0.063750 + (x2 * x2) * (-0.042727)$$

$$Y_3 = 1.331477 + x_3 * 0.017083 + (x3 * x3) * (-0.062727)$$

$$Y_4 = 1.331477 + x_4 * 0.552917 + (x4 * x4) * 0.069773$$

$$Y_5 = 1.331477 + x_5 * 0.093750 + (x5 * x5) * (-0.085227)$$

Fixing the coded levels of the 5 variables at -2, -l, 0, 1, 2, according to 5 equations above, the estimated yield values and effect sizes of five factors and levels showed in Table 4. From Table 4 the same conclusions as above can be obtained.

Table 4 Estimated yield and effect sizes of five factors and levels

Function	-2	-1	0	1	2	Maximum value
Y_1	1.2481	1.2975	1.3315	1.3500	1.3531	MAX (X_1 = -1.5790) =1.2708
Y_2	1.0331	1.2250	1.3315	1.3525	1.2881	MAX (X_1 = 1.4203) =1.3358
Y_3	1.0464	1.2517	1.3315	1.2858	1.1147	MAX (X_1 = -0.4147) =1.2984
Y_4	0.5047	0.8483	1.3314	1.9542	2.7164	MAX (X_1 = 2) =2.7164
Y_5	0.8031	1.1525	1.3315	1.3400	1.1781	MAX (X_1 = -1.7114) =1.2423

Optimization analysis: 28 pieces of combination after optimization analysis using SAS (SAS Institute Inc., 1999) based on the regression equation were obtained, after frequency analysis based on the 28 pieces of combination, the yield expectation was over 3.1%. Results of the analysis showed that the optimum technical condition was as follows: temperature was 100°C, extracting times was 4~5, time cost in every extraction was 1.4~1.8 hours, liquid/solid ratio was 1:18~1:20, acid concentration was 0.10~0.14mol/L.

Conclusions

1) *5 variables effected on yield be arranged with significance :* temperature > extracting times> time> liquid/solid ratio> acid concentration.

2) *Optimum technical condition was as follows:* temperature was 100°C, extracting times was 4~5, time cost in every extraction was 1.4~1.8 hours, liquid/solid ratio was 1:18~1:20, acid concentration was 0.10~0.14mol/L. The yield of soluble

polysaccharide by acid extraction from rapeseed meal was over 3.1% under this condition.

3) *Validate tests:* validate tests were done by selecting 5 combinations accidental from the optimum extraction parameter, results showed in Table 5.

Table 5 Validate test results

C(mol/L)	R(g/ml)	t(hour)	T(°C)	N	yield (%)
1	0.10 1:20	1.8	100	4	3.24
2	0.10 1:20	1.5	100	5	3.32
3	0.12 1:18	1.8	100	5	3.20
4	0.14 1:18	1.5	100	5	3.12
5	0.14 1:20	1.5	100	4	3.17

C- acid concentration; R- liquid/solid ratio; t- time cost in every extraction; T- temperature; N- times

Result of validate tests indicated that the yield of soluble polysaccharide by acid extraction from rapeseed meal can actually be over 3.1% under this condition.

References

Dong Hongxin, Lu Zuozhou(2004). Study on Soluble Polysaccharide by Acid Extraction from Pleurotus Ferulae.Acta Microbiologica Sinica **44**, 101-103.

Jiang Qiuyan, Qiao Xuguang, Zhang Zhenhua(2005). Effect of Extr action Conditions on the Yield of Neutral Polysaccharide from Garlic (Allium Sativum L). Journal of Chinese Institute of Food Science and Technology **12**,101-105.

Lin Yuman, Yan Chunsheng, Yu Ping(1998). Studies on Soluble Polysacchaide by Acid Extraction from Dictyophora duplicata—— Isolation, Purification and Identification of Dd—2DE.Jounal of FuJian Teachers University (Natural Science) **14**, 62-66.

Robert J. Redgwell, Carl E.Hansen(2000). Isolation and characterization of Cell Wall polysaccharides from cocoa beans. Planta **210**, 823-830.

Ruan Zheng. Su Jie et al(2005). Characterization and immunomodulating activities of polysaccharide from Lentinus deodes. International Immunop harmacology **5**, 811-820.

Yan Fengwei, Wu Moucheng, Jiang Hong (2004). Compositive extraction of rapeseed cake.Transaction of the CSAE **3**, 209-212.

Yang Aimei, Lei Hongxia.A(1998). Study on Mathematical Model for Optimum Cultivation. Acta Agricultural Boreali-Sinica **13**, 70-73.

Zheng Xiaodong, Zhou Min, Fu Chengxin(2000). Optimization of Polysaccaharides Ext ration f rom Coix Lachryma - jobi Kernel. Journal of the Chinese Cereals and Oils Association **15**, 19-22.

Preparation of peptides hydrolyzed from rape pollen glutelins

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Abstract

Rape pollen stored more than 1 year was used as material in this study. A research on protein fractionation of rape pollen defatted and cell-fragmentated was performed. Rape pollen gluntelin was hydrolyzed with alcalase and the crude peptides of rape pollen were obtained. The scavenging effect of rape pollen glutelin and its hydrolysate on hydroxyl free radicals(·OH) in the deoxyribose-iron system was studied. The results showed that glutelins and albumins were found to be the predominant proteins in rape pollen,comprising 55.7% and 39.0% of total proteins, While globulins and prolamins were 3.2% and 2.1%,respectively.The optimum conditions of alcalase enzymatic hydrolysis have been determined by mono-factor analysis and response surface methodology as follows:pH =9,hydrolyzing temperature 50°C,enzyme concentration 1460U per gram of substrate, concertration of substrate 6%,hydrolyzing time 2h.In the antioxidation test, the inhibition rate ·OH of glutelins was 25.1%. After being hydrolyzed, the inhibition ratio of ·OH of glutelins peptide was singnificently increased to 70.0%.

Key words: Rape pollen, Enzymatic hydrolysis, Preparation

Introduction

Rapeseed is one of the most important oilseed crops cultivated in the world, thus rape pollen is the cheap high-yielding pollen. However, because the rape pollen has a special unpleasant flavor that can not be accepted by young people, it is not populared by consumers directly as a commodity for sale,leading to large bulks of storage. Protein content is as high as 20-25% in Rape pollen, which is comparable to soybeans and peas,suitable proportion of its amino acid composition, with higher contents of essential amino acids, much peptide of higher quality physiological activity is expected to be obtained after the enzymatic modification.

Alcalase is a microbial protease from the bacterium *Bacillus licheniformis* with endopeptidase activity, which can hydrolysis protein to be short peptides. Response surface methodology(RSM) was originally described by Box and Wilson(5)as being effective for responses that are influenced by many factors and their interactions.

The authors have carried out some preliminary work on the protein extracted from the rape pollen and enzymatic modification with the rape pollen stored one year as raw material. And the old rape pollen is much more conducive to breaking cell. The initial findings showed : All the nutrient contents of new rape pollen is not much different from the old one.The protein contents in rape pollen account for 22.4% after being defatted and cell-fragmentated. Glutenin contents account for more than half of the contents of total protein, however, glutelin is alkali - soluble protein and its bioavailability in human gastric environment is very low, thus peptide obtained following the enzymatic modification with glutelin used as raw material, which not only improve its own bioavailability, but also enhance biological activity. activity of anti-hydroxyl radical has increased markedly after enzymolysis.Reactive oxygen species including free radicals lead to lipid peroxidation in organism, and initiate. inflammation, cancer, aging, atherosclerosis and other origin of diseases. Further in-depth study is expected to obtain the rape pollen peptide of the physiologic function including higher anti-aging,anti-fatigue, immunological regulation, etc., and provide a new health care products for human beings, and is conducive to multiple utilization of resources of staple agricultural products such as rape,imcrease the added value of agricultural products,and is helpful to solve the "three rural issues".

Materials and Methods

Materials

Rape pollen Wuhan small Bee Pollen company
Alkaline proteinase companies :Denmark NOVO companies (activity of 146,000 u/mL)
Sephadex G-25 (Pharnacial), Bactiracin(Sigma),Glutathione(Reduced, Amresco)

All other chemicals were of analytical grade.

Methods

The determination of the protein content of each component of Rape Pollen after being defatted and cell- fragmentated

Use different solvents to extract albumin, globulin, gliadin and glutelin, concentrate the protein solution obtained, and then use micro-Kjeldahl method (Moucheng Wu,2002) to determine the protein content of each component.

Total nitrogen determination:

Total nitrogen was determined according to the micro-Kheldahl method, crude protein content was calculated using a conversion factor of 6.25.

Measurement of Degree of hydrolysis:

The degree of hydrolysis, defined as the ratio of amino nitrogen/total nitrogen(AN/TN), was calculated according to the methods of X. H. Zhao (8,9).

$$DH\% = AN(\text{amino nitrogen}) / TN(\text{total nitrogen}) \times 100\%$$

The AN, produced by hydrolyzing, was determined with formaldehyde titration procedure, While total nitrogen was determined according to the micro-Kheldahl method above(1.2.2).

Determination of the free amino acid content Ninhydrin chromatometry method (he sheng li, 2000)

The amino acid analysis of rape pollen protein isolates

HPLC analysis of amino acid composition.(Institute of the Chinese Academy of Agricultural Sciences, Wuhan Fuel Testing Center)

Determination of •OH inhibition ratio

•OH inhibition ratio is determined by Halliwell,etc (Halliwell et al.1987) and the method is to be improved. Referring to Halliwell (1987) method: The reagents added to a clean cuvette in order are as follows 0.4 mL of 50mmol·L^{-1} KH_2PO_4-KOH buffer (pH 7.5),0.1mL sample of a certain concentration, 0.1mL 1.04mmol·L^{-1} EDTA,0.1 L10mmol·$L^{-1}H_2O_2$, 0.1mL 60mmol·L^{-1}deoxyribose (not in the comparison),0.1mL 2mmol·L^{-1}VC,and 0.1mL 1mmol·L^{-1} FeCl3. every **c**uvette of the final volume is 1.0 mL. incubated at 37 ° C for one hour before removal, adding 1ml 25%HCl,terminating reaction,In addition,adding 1.0mL1%TBA solution to blend solution in each cuvette, then cooked in boiling water bath for 15 min. Cooling immediately after centrifugation (3000r/min). Measuring the absorbance at 532 nm, and calculating according to the following formula :

• OH inhibition rate (%) = [(A0 - A) / A0] × 100 (1-1)

where A0- not joined the absorption inhibitor •OH

A value-adding the inhibitor optical • OH

Preparation of rape pollen defatted and cell-fragmentated

After the impurities in rape pollen and the molded pollen grain being removed, grinding full,refrigerating at -10 degrees above freezing for 24 hours, stirring in the hot water with 80 °C,immediately cooled to 40 —45 °C and stirred at this temperature for extraction for 8 ~ 10 h.then freeze drying until rape pollen of which cell have been broken be obtained (Zhi Liu, 1989). Pollen obtained will be soaked with petroleum ether after the grinding, then placed in the fume cupboard for ventilation for 12h,recirculated and condensed in the water bath at 55 °C for 8h, filtrating, placed in the fume cupboard for ventilation for one hour, then vacuum dried at constant temperature of 45 °C.defatted pollen of which cell has been broken will be obtained.Pollen has been dried is put into the brown bottle, and placed in the shady place for reserve(Adnan et al., 2000).

Preparation of pollen protein isolates

The electrostatic repulsion of protein between particles in the electrostatic state is the smallest,therefore the solubility is minimal,The isoelectric points of proteins are different,thus adjusting the solution pH at or near the isoelectric point of a certain protein can be used for protein precipitation so as to achieve the purpose of purification, then extracting.

Nutritive value evaluation of pollen protein isolates

Evaluating essential amino acids (EAA) with the method of amino acid ratio coefficient in the non-biological evaluation method (Sheng-Tao Zhu and Wu Kun,1988). calculating the following index with the standard the World Health Organization (WHO) and UN Food and Agriculture Organization (FAO) proposed, aimed at the recommended values of the essential amino acids (E. C. Henley;J. M. Kuster, 1994)for the children aged 10-12 :amino acid ratio (ratio of amino acid, RAA).amino acid ratio coefficient (the ratio coefficient of amino acid.RC) and the amino acid ratio coefficient pm (Score of RC, SRC).Formula is as follows : RAA=the content of the essential acid in rape pollen/the RDA of the essential acid

RC=RAA/the average of RAA

CV=the standard deviation of RC/ the average of RC

SRC=100-CV*100

If the amino acid composition of food is consistent with the recommended values of amino acids, then RC = 1. RC>1 or RC<1 demonstrate that deviating from the amino acids mode.RC>1 demonstrate that the amino acids is relatively surplus,RC <1 demonstrate that this amino acids is relatively insufficient ; amino acid of which RC is the minimal is the first limited amino acid.

Enzymolysis reaction of alkali-soluble glutelin

Collocating alkali - soluble protein solution of a certain concentration,heating the solution to temperature of test set in waterbath,adjusting ph to the ph of test set,adding 0.25mL alkaline proteinase and stirring at lower rate.maintaining the stability of ph by adding diluted acid and alkali once every half hour.after 2 hour's Hydrolysis, enzymolysis solution is placed in the boiling water for the 10 min for the deactivation of protease, reaching the condition of metered volume after

cooling, filtrating, the filtrate is to be placed in the fridge for analysis。

Optimization of hydrolytic conditions

A three-factor central composite design was employed to examine the response,degree of hydrolysis (DH%) of glutelin by alcalase as changed with the independent variables, the substrate concentration, (%,X_1) temperature(°C,X_2)and pH value (X_3).A quadratic polynomial regression model was assumed for prediction the response. Every factor (Code X1 to X3) had three levels which corresponding to three code values.There were total 15 experiments conducted. In every experiment levels of the factors were arranged according to Table 1. The model proposed is described in Table2, under results and discussion, experimental data were analyzed for response surface regression for a quadratic polynomial model using SAS software (SAS Institute Inc.1990)

Table1 Design of factors levels in experiment

Factor	Code	Code value	Level
		+1	4
[S]/%	X1	0	5
		-1	6
		+1	40
T/°C	X2	0	50
		-1	60
		+1	8
pH	X3	0	9
		-1	10

Table2 Different levels of factors arranged in experiments

Test number	Code value of experiment		
	X1	X2	X3
1	-1	-1	0
2	-1	0	-1
3	-1	0	+1
4	-1	1	0
5	0	-1	-1
6	0	-1	+1
7	0	+1	-1
8	0	+1	+1
9	+1	-1	0
10	+1	0	-1
11	+1	0	+1
12	+1	+1	0
13	0	0	0
14	0	0	0
15	0	0	0

Results and discussuion

Distribution of protein component in rape pollen defatted and cell-fragmentated

Table 2-1 distribution of protein component in rape pollen

categpry	Distribution of proteins in rape pollen defatted and cell-fragmentated (%)	Distribution in total protein (%)
albumin	7.769	39.0
globulin	0.4310	3.20
alcohol-soluble protein	0.6300	2.10
alkali-soluble protein	11.11	55.7
total	22.42	100

The results can be got from Table 2-1: albumin and alkali - soluble protein are the major protein in pollen,accounting for more than 90% of pollen's total protein,which is the main component composing the pollen protein.in addition, it contains a small amount of globulin and prolamine accounting for approximately 5% of total protein.

Nutritive value evaluation of pollen protein isolates

The results can be got from Table 2-2, lys's and Tyr+Phe's amino acid ratio coefficient of albumin, globulin, glutelin, and Thr's amino acid ratio coefficient of glutelin are all more than one (RC> 1).it illustrates that these amino acids are relative surplus.As the result of Met ant Trp were not measured, these two amino acid were not taken into consideration in the process of evaluation. the RC of leucine in albumin and globulin are the lowest,thus leucine is the first restrictive amino

acid of albumin and globulin. the RC of isoleucine in alkali - soluble protein is the lowest, **so** isoleucine is the first restrictive amino acid of glutelin.Amino acid ratio coefficient (SRC) is to evaluate the protein quality by dispersion that all essential amino acids diverge from the amino acid model,SRC is highly correlated with the biological value and also is closer to biological value in number. it also indicates that from the table 2-2 the SRC of the glutelin is the highest, therefore its nutritional value is the best, and although the albumin is of the high content,its nutritional value is Almost not too much. alkali - soluble glutelin in human gastric acid environment is of very low bioavailability, therefore, we use glutelin as the experimental material for the follow-up experiments,and then enzymatic modification not only can increase bioavailability,but also increase biological activity.

Table 2-2 amino acid ratio of albumin, globulin, glutelin, amino acids Coefficient ratio and distribution of amino acid ratio coefficient (content of alcohol-soluble protein is little)

	Ile	Leu	Lys	Met+Cys	Trp	Tyr+Phe	Thr	Val	average	SRC
Ref value (10~12y)	2.80	4.40	4.40	2.20	0.90	2.20	2.80	2.50		
albumin										-0.20
RAA	0.100	0.093	0.825	0.068	—	0.286	0.343	0.152	0.267	
RC	0.375	0.348	3.090	0.255	—	1.071	1.285	0.569	1.000	
globulin										18.67
RAA	0.082	0.073	0.543	—	—	0.273	0.161	0.164	0.216	
RC	0.380	0.338	2.514	—	—	1.264	0.745	0.759	1.000	
glutelin										42.25
RAA	0.354	0.486	1.121	—	—	1.827	0.918	0.776	0.914	
RC	0.387	0.532	1.226	—	—	1.999	1.004	0.849	1.000	

Experimental results of enzymatic hydrolysis condition

Experiments are conducted in a random order for three repetition,the average of absorbance obtained in the experiment will be analyzed through SAS RSREG (Response Surface Regression) program, and response surface analysis graph and variance analysis table will be obtained.

Table 2-5 Parameters estimated in regression models (RSM analysis)

Parameter	a_0	a_1	a_2	a_3	a_{11}	a_{22}	a_{33}	a_{12}	a_{13}	a_{23}
Value in model about degree of hydrolysis	29.39	0.21	1.00	3.30	-4.05	1.46	-12.04	-3.54	1.08	-5.86

Table 2-6 The predicted value of regression model and experimental results

Response variable		Degree of hydrolysis	
[S]/%	T/°C	pH	%
6.20	49.8	8.69	17.8

Table 2-7 variance analysis of The regression equation

source of variance	Degree of freedom	Sum of square	FValue
Model	9	989.637	8.17*
Error	5	984.612	
Correct total	14	1051.537	
Linearly dependent coefficien	0.9364		

**$f_{0.01}(9,5)=10.2$; *$f_{0.05}(9,5)=4.8$

Results of 15 experiments were in table 3.Content of Hydrolysis Degree (DH) were used as response values in analysis of response surface regression(RSREG). The equation $DH(Y) = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3$ was used as regression model. The procedure RSREG of SAS also gave values of parameter estimated (Table 2-7). SAS RSREG procedures calculated using the regression equation coefficients (Table 2-3) on the basis of the mathematical analysis of the regression model, the results shown in table 2-4.Further analysis and regression model to estimate the value of more than f0.05 model F (9, 5).linear reached 0.9364 (Table 2-5),explain**ing** this regression equation to describe the relationship between the various factors and response,with all of its variable linear relationship between variables is notable that this method is reliable. The results of the regression model can be used to analyze and forecast. Alcalase endoprotease alkaline hydrolysis of insoluble protein substrate concentration, the optimal reaction conditions : 6%.pH 9.0, the temperature was 60 °C, compared with the end of 1460U/g protein,enzyme hydrolysis time 2h.

Studies on each pollen protein isolates and antioxidant activity of glutelin enzymolysis peptide.

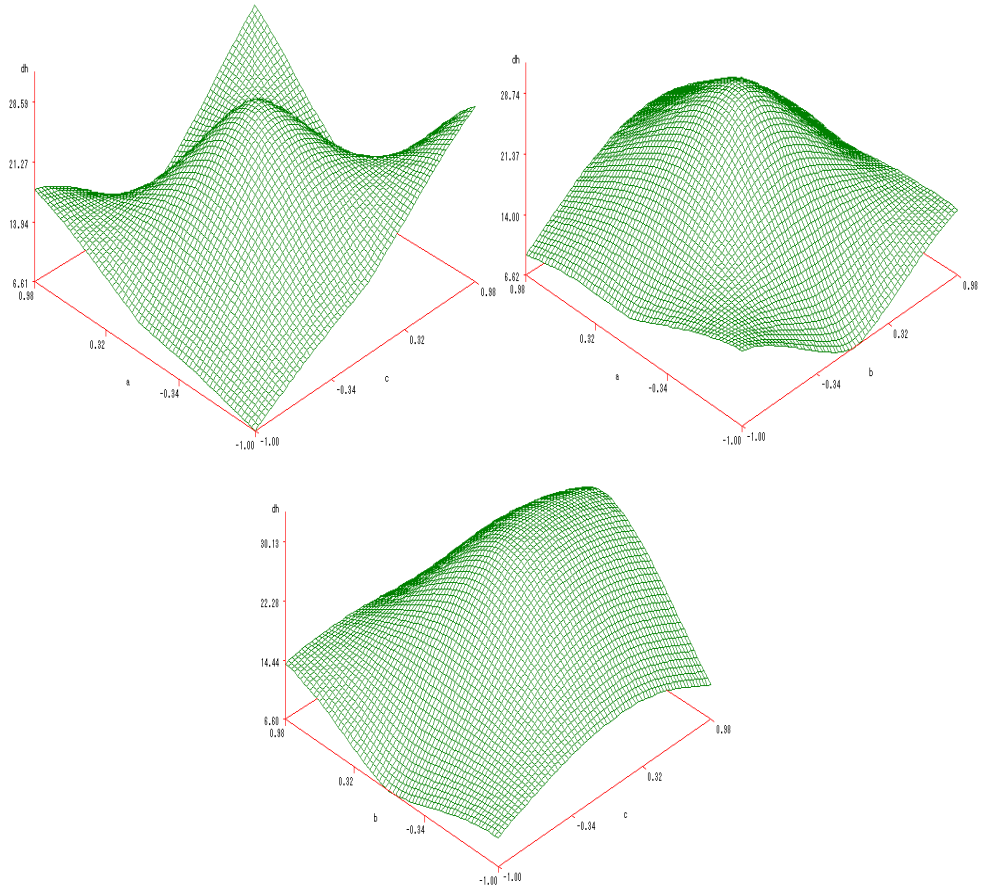

Fig.1 Response surface diagrams of Degree of hydrolysis

Biologically active peptide with low molecular weight**, having a strong antioxidant capacity,** will be formed after Pollen porotein is hydrolysed with protease.The results of. Table 2-8 show that the pollen alkaline protease rate of inhibition on OH is 25.08% before hydrolysis.While being hydrolysed it can got to 70%. It demonstrates that the activity of pollen alkaline protease improve significantly after hydrolysis.

Table 2-8 The hydroxyl radical inhibition rate of each pollen protein isolates and glutelin enzymolysis peptide. (%)

concentration (mg/ml)	2	2	2	2
Sample	albumin	globulin	glutelin	glutelin enzymolysis
The inhibition rate (%)	54.35	45.03	25.08	70.0

Conclusion

The results showed that glutelins and albumins were found to be the predominant proteins in rape pollen, comprising 55.7% and 39.0% of total proteins.While globulins and prolamins were 3.2% and 2.1%, respectively. The optimum conditions of alcalase enzymatic hydrolysis have been determined by mono-factor analysis and response surface methodology as follows:pH =9,hydrolyzing temperature 50°C,enzyme concentration 1460U per gram of substrate, concertration of substrate, 6%,hydrolyzing time 2h.In the antioxidation test,the inhibition rate ·OH of glutelins was 25.1%.After being hydrolyzed,the inhibition ratio of ·OH of glutelins was singnificently increased to 70.0%. It demonstrates that the activity of pollen alkaline protease improve significantly after hydrolysis.

References

Olsman H. Hydrolyzed vegetable proteins as functional food and autolyzed in gredi- ents. Oil Chemists' s Soc. 1979.56 : 375-376.

Chengqing Kai, huiyuan Tao Kenema. Isolation of bitter melon polypeptides and its hypoglycemic activity Wuxi light University Journal, 2005,24 (1) : 49-51

Chun Ling Zhang, shu wang,li Cao and others. The stimulating and immunizing effect of pollen bioactive peptides.New Drug magazine 1999,8 (7) : 453-455

E. Decker,A D. Crum. Inhibition of oxidation by carnosine rancidity insalted ground pork. Food Sci..1991, 56 : 1179

Elder M. C., A. M. Fernandez.Lassota et al.. Inhibition of tubulin polymerisation by marine vitilevuamide a bicyclic peptide at a site distinet from colchicines.the dolastatin and vinca alkaloids. cobrotoxin Dubois.2002, 64 (4) :A V-707

Fang Qian, Yan Tang, fengyi Wang.studies on Alkaline endoprotease hydrolyzing soy bean protein.Dalian Light Industry Journal, 2000,19 (1) : 40-44

Feng Song Cong.Biochemical experiments.1 edition.Shanghai : Shanghai Jiaotong University Press, 2005:111-118

Hao sun,xingwang cai.the premium condition for alkaline proteinase hydrolyzing casein.Dalian Institute of Light Industry Journal, 2003,22 (1) : 25-27

Hui He, Bijun Xie, Zhuo Yang. Studies on hydrolysed peptide of Corn and soybean protein and its activity.Cereals, Oils and Foodstuffs technology, 2002,10 (1) : 14-16

J. Adler. Enzymic hydrolysis of food proteins. Elsevier Applied Science Publishers,A 1986:l-8

Jiangguo Pan, Yao Zheng, Yuan Hong Liu.Discussion on the function factor in pollen.China naturally medical journal, 2001,8 (3) : 164-166
Jiaxi Xu, yanling Song..Studies on Several pollen polypeptide and polysaccharide.Journal of Beijing University, 1999,35 (2) : 178-182
Juhua xu Bee Pollen's effect on human health.Chinese food and nutrition, 2004,11:54-58
Ling Peng Pei, Ying Wu.study progress of polyPeptide.Capital Normal University, 2004,3 (25) : 56-60
Mukhin.L Enzymatic hydrolysis of proteinsfrom crustaceans of the barents sea. Applied Biochemistry and Microbiology,37 (5) : 538-542
Tao Feng, Dong-Xu Cao.Response surface method used to determine the optimum extracting conditions of flavonoids in bamboo leaves.Journal of Tianjin Institute of Light Industry, with Ye Xu 2003,2:9-13
Wen Huang, Bijun Xie.Studies on ginkgo protein separation, purification, its physical and chemical properties and its antioxidant activity.China's agricultural science, 2004,3 (10) : 1537-1543
Xinhuai Zhao, Zhibiao Feng. Determination of the degree of hydrolysis of protein hydrolysates.Food Science, 1994,11:65-67
Yihong bao,hejing sheng. the protein and polypeptide Preparation of pecans and the hydroxyl free radical scavenging.Food Science, 2005,26 (9) : 515-518
Yongfang Peng, Chang-hong Yang. The chemical composition analysis of Pollen.Analytical Journal, 1998,4 (17) : 34-36
Yongfang Zhao. Technology and Application of Biochemistry.The third edition.Beijing : Science Publishing House : 101-123
Yuhong ren,yupeng liu. Bee pollen's function factor.Food research and development 2001,8 (22) : 44-47
Zhaohui Xue. Preparation of Rapeseed peptide and studies on its biological activity.[PhD thesis].Wuhan, Huazhong Agricultural University Library 2004

Standardization of reaction kinetics for enzymatic hydrolysis of Indian mustard seed oil for extraction of Erucic acid

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Abstract

Erucic acid (EA) constitutes about 30-51% of the total fatty acids of Indian mustard (*Brassica juncea* L. Czern. & Coss.) seed oil. It has got many industrial applications. Hydrolysis of mustard oil by commercial lipase enzyme, Lipozyme TL-100L (source: Novozymes) was investigated for the extraction of EA. The free fatty acids (FFA) present in the mustard seed oil were removed by repeated washing with 90% aqueous ethanol before performing hydrolysis. Hydrolysis was performed at a temperature of 55 ^{0} C and pH of 5-6 in a water bath shaker for different enzyme concentrations (0.01, 0.02, 0.5, 1.0 and 3.0%) and time periods (30min, 1hr, 3hr, 6hr and 24hr). Released fatty acids were extracted with $NaHCO_3$. This was simple, easy and gave comparatively more recovery of EA. Percentage of FFA as oleic analyzed for different hydrolysis time intervals of 1hr, 2hr, 3hr, 4hr, 5hr, 6hr and 24hr and was minimum at 0.3% and maximum at 0.01% as 1.17 and 4.32 respectively for a time period of 24hr. Percentage of EA in the released acid fraction was analyzed by Gas Chromatography. The analysis revealed that EA content showed a reduction with an increase in the concentration of lipase enzyme used for hydrolysis after threshold equilibrium. EA content was found to be 81.50% (30min); 84.30% (1hr); 90.05% (3hr); 99.30% (6hr); 88.63% (24hr) for 0.01% and 79.59% (30min); 86.49% (1hr); 82.56% (3hr); 80.01% (6hr); 69.43% (24hr) for an enzyme concentration of 0.05%. As lipase, TL-100L is 1, 3 specific therefore with more concentration of enzyme the percentage of EA hydrolysis was decreasing as it started hydrolyzing other relevant positions in triglyceride chain after a certain time period and percentage. It was observed that enzyme concentration of 0.01% for a period of 6hr was the ideal parameter for enzymatic erucic acid extraction from mustard oil.

Key words: Erucic acid, lipase, mustard seed oil, hydrolysis

Introduction

Erucic acid (EA) is 22-carbon monosaturated fatty acid with a single double bond at the omega 9 position. EA constitutes about 30-51% of the total fatty acids of Indian mustard (Kaushik and Agnihotri, 2000). EA has various industrial applications from lubricants to slip agent in plastic industry. The primary use of EA is a precursor to another chemical erucamide. Erucamide is used in the processing of plastic film, particularly for film made from polymers, polyethylene and polypropylene. The market for erucamide along with other important uses for EA such as personal care products and lubricants derives an annual world demand to a great extent. Therefore EA extracted from mustard oil will be an alternative to many petrochemical products. Till now high pressure fat splitting of mustard oil has remained in practice but recently with the advancement in the field of biotechnology this focus has shifted towards the 'Green chemistry', i.e. biocatalysts. The enzyme-mediated hydrolysis is an alternative to tedious and expensive high-pressure methods.

Lipases, triacyl glycerol hydrolases are an important group of biotechnologically relevant enzyme and their applications in several areas of biotechnology has been well studied (Gupta et al., 2004). This group of enzymes can catalyze the hydrolysis of fatty acid ester bond in triacyl glycerol thus releasing FFA under aqueous and non-aqueous conditions as well. Therefore, the enzyme lipases are the best fit enzymes for the hydrolysis of mustard oil. The role of lipase in lipid metabolism is widely studied (Gordillo et al., 1998; Knezevic, 2004; Gomez et al., 2005; You and Baharin, 2006; Srivastava et al., 2006). Lipase hydrolysis of mustard oil under water limited conditions yield dierucin, which can be easily isolated (Kaimal et al., 1993). EA forms 25.64% of the total fatty acid composition in a study of mustard seed oil by commercial lipase from *Candida rugosa* (Tinoi, 1999). EA content in the FFA reaction reached 82.2% with a recovery of 71.7% during enzymatic hydrolysis of Crambe oil using porcine pancreatic lipase (Tao and He, 2005). Partial hydrolysis of triacyl glycerols of high EA seed oils catalyzed by lipases from *Candida cylindracea* and *Geotrichum candidum* leads to enrichment of EA and other very long chain monounsaturated fatty acids in the acylglycerols while the C_{18} fatty acids are enriched in the free fatty acid fraction (Mukherjee and Kiewitt, 1996). In this study EA isolation from mustard seed oil was evaluated using enzymatic hydrolysis.

Materials and methods

Commercially refined mustard seed oil was used for the hydrolysis analysis. Lipozyme TL 100 L (Source: *Thermomyces longinosus*) was received from Novozymes A/S (Bagsvaerd, Denmark). The FFA of mustard oil were removed by repeated washing with aqueous ethanol in the ratio of 10:90 before hydrolysis. The enzyme concentrations used were 0.01, 0.02, 0.5, 1.0 and 3.0%. The time parameters were 30min, 1 hr, 3hr, 6hr and 24 hr. pH of the reaction medium was maintained at 5.5. Hydrolysis was performed at 55^{0}C in a water bath shaker. Solvents were either of HPLC grade or AR grade.

Analysis of % oil hydrolysis: Percent oil hydrolysis was estimated titrimetrically by comparing the alkali (0.1N KOH) used to neutralize the released fatty acids. The results were expressed as percentage of FFA as oleic acid = 28.2x N x V /W, N

= Normality of KOH solution, V = Volume of KOH solution used (ml), W = Weight of the sample (gm).

Extraction of EA: EA was isolated from the hydrolysis mixture through two different approaches. viz. Solvent extraction using aqueous ethanol and $NaHCO_3$ extraction.

1) Solvent extraction using aqueous ethanol: Released fatty acids from mustard oil as a result of enzymatic hydrolysis were extracted with solvent extraction approach. Aqueous ethanol in the ratio of 30:70-10:90 was used to isolate EA. Separation was carried out by separating funnel. After thorough washing with 90% aqueous ethanol, the ethanol extract was evaporated and used for fatty acids profile estimation.

2) Na HCO_3 extraction: At the completion of reaction, $NaHCO_3$ was added for increasing the pH and for transferring the released fatty acids in to the aqueous phase. It was followed by adding 1 volume of fat solvent. Resultant was centrifuged to 10,000 g to pellet out the fatty acid salt and to remove the oil. Pellet was washed twice with fat solvent. After evaporating the fat solvent pellet was resuspened in 10 ml of distilled water and its pH was adjusted to 2 with the help of 0.1 N HCl and 2 volumes of fat solvent was collected and was concentrated by evaporation.

Estimation of hydrolysis products: Fatty acids estimation was done with the help of Gas Liquid Chromatography (GLC). Fatty acids were converted to their respective methyl esters by using 0.5 N methanolic potassium hydroxide before GLC analysis in heptane as solvent medium.

Fatty acid esterification: 1 ml of 0.5 N methanolic potassium hydroxide was added to 15 ml fatty acids solution dissolved in n-heptane and was vigorously shaked for 5 minutes and was kept stand by for next 5 minutes for phase separation. Heptane layer was collected and two washes with distilled water were given to remove the traces of KOH from it. Heptane was evaporated to 1/5 th volume at 70 ^{0} C.

GLC analysis: GLC analysis of fatty acid methyl ester was done at oven temperature of 130^0C and column temperature 230^0C with FID having nitrogen as a carrier at a pressure of 4 kg per cm^2 and 0. 2 µl of methyl ester was injected for analysis.

Statistical analysis: The experiments were performed in ten replicates and the statistical analysis was done using the ANOVA test in the Stat plus package.

Results

Hydrolysis of mustard seed oil with lipozyme TL 100 L at various time periods and enzyme substrate ratios have been depicted in the Fig.1. Kinetics of mustard oil hydrolysis was performed for different enzyme concentrations (0.01, 0.02, 0.5, 1.0 and 3.0%) and for the reaction time periods of 1, 2, 3, 4, 5, 6 & 24 hr. The percentage of FFA had increased from 5.85 to 10.46, when the amount of enzyme used was 0.01% and 3.0% respectively for a reaction period of 5hr. Percentage of FFA released after hydrolysis was maximum at an enzyme concentration of 0.01% and minimum at 3% i.e. 4.32 and 1.17 respectively for a time period of 24hr. Isolation of EA from the fatty acids released as a result of enzymatic hydrolysis of mustard seed oil was done using two extraction methods. Extraction methods were analyzed for different enzyme concentrations (0.01, 0.02, 0.5, 1.0, 3.0%) and reaction time periods (30 min, 1, 3, 6 & 24 hr). EA content in the free fatty acids fraction reached to 93.21% and 99.30% after a reaction time period of 6hr and 46.00%, 88.63% for a reaction time periods of 24hr with an enzyme concentration of 0.01% for solvent extraction and $NaHCO_3$ precipitation respectively (Fig. 2). Excess enzyme did not contribute to the increase in EA content in the FFA fractions in both the approaches (Table 1 & 2).

Discussion

The extent of hydrolysis of mustard oil by lipozyme was found to be influenced by the enzyme substrate ratios and the reaction time periods. Both the time course and the enzyme concentrations gave an insight into the performance of an enzyme on the reaction progression. It was found that percentage of FFA released was maximum at a reaction period of 5 hr for the entire enzyme concentrations studied. Degree of hydrolysis found to be lower in low enzyme substrate ratio and found to be increasing linearly with an increase in enzyme concentration. There was a significant reduction in the FFA production in the later stages of hydrolysis and it was due to enzyme denaturation by increase in pH with the result the reaction attained equilibrium. During the course of enzyme catalyzed reaction an increase in product concentration can inhibit enzyme activity (Worthing, 1997). EA recovery from released fatty acids was achieved successfully in both extraction methods. But among these approaches $NaHCO_3$ precipitation is simple, easy and gave comparatively more recovery of fatty acids. Solvent extraction using aqueous ethanol also gave higher percentage of EA in the FFA fraction. Typical solvents used for FFA extraction include, methanol, ethanol and isopropyl alcohol (Kim et al., 1988). Crystallization of EA from the lipase catalyzed hydrolyzed mixture of mustard seed oil was done with aqueous ethanol with 34% purity (Tinoi et al., 2000). But main problems with solvent extraction is its time consuming and tedious process. Through solvent extraction, F.F.A and neutral lipids can not be separated completely (Worthing, 1997). As lipase is considered as 1,3 specific therefore with more concentration of enzyme the percentage of EA is decreasing in the FFA fraction as it starts hydrolyzing other relevant positions in triglyceride chain after a certain time period and percentage. Enzyme concentration of 0.01% for a period of 6hr is working well for both the approaches.

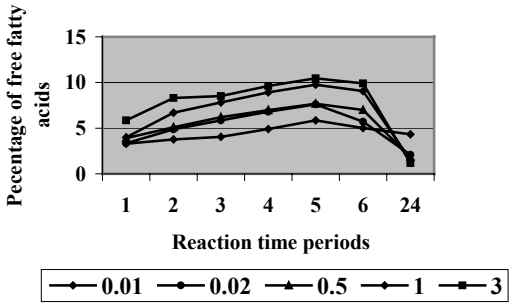

Fig. 1. Hydrolysis course of mustard seed oil with Lipozyme TL 100 L at different reaction time periods (hr) and enzyme concentrations (0.01%, 0.02%, 0.05%, 1.0% & 3%).

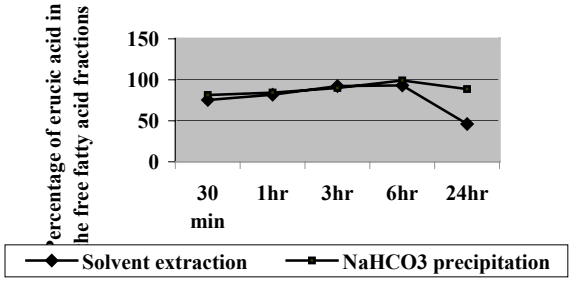

Fig. 2. Percentage of erucic acid in the free fatty acid fractions in different extraction approaches for different time periods. Hydrolysis was performed with an enzyme concentration 0.01% at 55 ^{0} C and moisture 25% v/v.

Table 1. Fatty acid composition of mustard oil after hydrolysis for a time period of 6hr at different enzyme concentrations (Extraction method: $NaHCO_3$ precipitation)

Enzyme concentrations used	Oleic C $_{18:1}$	Linoleic C $_{18:2}$	Linolenic C $_{18:3}$	Erucic C $_{22:1}$
0.01	0.00	0.00	0.00	99.30
0.02	0.46	0.35	0.35	96.44
0.05	16.61	1.54	0.00	80.01
1.00	5.04	4.01	6.16	76.11
3.00	12.86	12.82	5.34	63.04

Table 2. Fatty acid composition of mustard oil after hydrolysis for a time period of 6hr at different enzyme concentrations (Extraction method: Solvent extraction using aqueous ethanol)

Enzyme concentrations used	Oleic C $_{18:1}$	Linoleic C $_{18:2}$	Linolenic C $_{18:3}$	Erucic C $_{22:1}$
0.01	0.00	0.24	2.80	93.21
0.02	0.55	0.77	0.74	92.81
0.05	0.83	0.23	3.36	89.53
1.00	1.77	3.24	12.64	75.80
3.00	8.30	3.77	10.12	67.68

Conclusion

Reaction temperature at 55 ^{o} C, 25% (v/v) water content for a period of 5 hrs is optimum for the Lipozyme TL 100 L enzymatic hydrolysis of mustard seed oil for the production of FFA. $NaHCO_3$ precipitation is found to be more suitable extraction method for the recovery of EA than the solvent extraction.

Acknowledgement

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References

Gordillo, M.A., Montesinos, J.L., Casas, C. (1998). Improving lipase production from *Candida rugosa* by a biochemical engineering approach. Chemistry and Physics of Lipids 93, 131-142.

Gomes, F. M., Silva, G. S., Pinatti, D. G., Conte, R. A., De, H. F. (2005). Wood celulignin as a alternative matrix for enzyme immobilization. Applied Biochemistry and Biotechnology 121, 255-268.

Gupta, R., Gupta, N., Rathi, P. (2004). Bacterial lipases: an overview of production, purification and biochemical properties. Applied microbiology and Biotechnology 64, 763-781.

Kaimal, T. N. B., Prasad, R. B. N., Rao, C.T. (1993). A novel lipase hydrolysis method to concentrate Erucic acid glycerides in Cruciferae oils. Biotechnology Letters 15, 353-356.

Kaushik, N., Agnihotri, A. (2000). GLC analysis of Indian rapeseed-mustard to study the variability of fatty acid composition, Biochemical Society Transactions 28, 581–583

Kim, S.K., Kim, C.J., Cheigh, H.S., Yoon, S.H. (1985). Effect of caustic refining, solvent refining and steam refining on the deacidification and colour of rice bran oil. Journal of American oil chemists society 62, 1492-1495.

Knezevic, Z. (2004). Immobilizes lipases as practical catalysts. Beograd (Serbia and Montenegro) Zaduzbina Andrejevic. Monograph.

Mukherjee, K.D., Kiewitt, I. (1996). Enrichment of very long chain mono- unsaturated fatty acids by lipase catalyzed hydrolysis and transesterification. Applied microbiology and Biotechnology 44, 557-562.

Srivastava, A., Akoh, C. C., Chang, S. W., Lee, G.C., Shaw, J.F. (2006). Candida *rugosa* Lipase LIP 1- catalyzed transesterification to produce human milk fat substitute. Journal of Agricultural and food chemistry 54, 5175-5181.

Tao, C., He, B.B. (2005). Enzymatic Isolation of erucic acid from HEA oils, with biodiesel as a by- product: Preliminary investigation. American Society of Agricultural and Biological Engineers.

Tinoi, R. (1999). Isolation of Erucic acid from Mustard seed oil by *Candida rugosa* lipase. M.Sc. Thesis. Chiang Mai University, Thailand.

Tinoi, R., Niamsup, H. and Rakariyatham, N. (2000). Isolation of Erucic acid from mustard seed oil by lipase hydrolysis and crystallization. Chiang Mai Journal of Science 27, 89-101

Worthing, W. (1997). Incorporation of novel fatty acids in to canola oil using lipase from *Mucor miehei*. M.Sc. Thesis. University of Guelph. p. 59.

You, L.L., Baharin, B.S. (2006). Effects of enzymatic hydrolysis on crude palm olein by lipase from Candida rugosa. Journal of food lipids 13, 73.

Research and application of twin-screw expeller for cold pressing of de-hulled rapeseed

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Abstract

In order to solve the technical problems of cold pressing of de-hulled double-low rapeseed, a new twin-screw expeller with a principle of combination of joggling and non-joggling was designed and manufactured. The pressing chamber had characteristics of multiple-stage compression and relaxation and a thin layer of materials when pressing. Theoretical compressing ratio of the twin-screw expeller is 23.0, and ratio of length and diameter of pressing chamber is 11.5 with a pressing time of 180 seconds. In the twin-screw expeller, materials could be clear intensely so that the problem of de-hulled rapeseed feeding was solved. The twin-screw expeller was applied to cold pressing of de-hulled rapeseed successfully. The oil content of cold pressing cake is reduced to about 15%. Cold pressing oil approaches the standard of the third grade rapeseed pressing oil (Chinese national standard, GB1536-2004). Cold pressing cake temperature is lower than 70°C which assures good quality of oil and cake. Twin-screw expeller is suitable for pressing de-hulled rapeseed with obvious quality improvement of rapeseed oil and reduction of energy consumption, which can increase processing value of double-low rapeseed.

Key words: twin-screw expeller, double-low rapeseed, de-hulled, cold pressing, equipment

Introduction

At present, pre-pressing process is mainly used to process double-low rapeseed in the world, which is carried out with hulled rapeseed at high temperature for long time. As a result, the quality of crude oil is not good, for example, dark colour, rich in impurities, difficult to refine (Elizabeth et al., 1991). The cake is contains low protein, with dark colour and bad taste. Moreover, protein is over-disnatured, and effective amino acids are badly destroyed, which affects the utilization efficiency of protein (Zheng, 2001). Study on dehulling and cold pressing technology is gaining increase interesting all over the world. By using dehulling and cold pressing technique, perfect cold pressing oil could be produced by double-low rapeseed, and refine process is simplified (Huang, 2002). Meanwhile pollution by chemical reagents is avoided, and functional components such as VE can be retained in oil. Thus, this is an ideal method to provide natural green food. With this process, the protein content in cake is improved, and effective amino acids are not destroyed under lower temperature. The nutritional value of crude protein is also improved. At the same time, the process of flaking and cooking can be skipped, which obviously reduces energy consumption (Ragnar, 1992; Rasehorn et al., 2000).

Cooperating with University - Gesamthochschule Essen, Cimbria Sket GmbH had made key equipment used for dehulling and cold pressing process in 2000. Rapeseed dehulling and cold pressing pilot-plant has been built, cold pressing oil that is organic can be produced. The price of organic oil is 3~5 times more compared to rapeseed salad oil (Xin, 2005). Equipments used to dehull and separate rapeseed have been made at home (Zhang, 2004; Huang et al., 2000). And it is designed with many principles. Study on cold pressing equipment has just started (Li et al., 2004). However, the equipment used to cold press and produce oil with high efficiency should be exploited and applied widely. In this work, the study on the twin-screw expeller and its application is reported.

1 Problems of single-screw expeller

Experiments about cold processing of de-hulled rapeseed were started with the traditional single-screw expeller including ZX10, ZX18, ZY24. The result revealed that the feedstock was difficult to push in the chamber, producing shapeless cake and a little oil or even no oil. The possible reasons were assumed as following. Firstly, oil content in de-hulled rapeseed kernel amounted to about 45%, and the crude fiber reduced vastly to 3%~5%. So the properties of feedstock such as density, frictional factor, elastic modulus, etc. were obviously different from non de-hulled rapeseed oil, especially the friction among feedstock particles, or among feedstock particle and pressing cage inter surface reduced vastly. So it is difficult to deliver the feedstock in the chamber, and the pressure in the whole pressing progress was difficult to build up. Secondly, in cold pressing, the de-hulled rapeseed was not pretreated by flaking and cooking, and the feedstock cells were basally integrated. The affinity between protein and fat was still strong. So, higher pressure was needed to press the oil out. Traditional single-screw expeller was difficult to realize cold pressing of de-hulled rapeseed.

The structure of the single-screw expeller made in China was evaluated (Zhu & Shi, 1991). Many problems were detected, such as too small ratio of length and diameter of pressing chamber, a little low total theoretical compressing ratio, too short length covered by feedstock delivering screw, low delivering ability, and so on. The chamber of present single-screw expeller was not suitable for cold pressing. In order to realize the cold pressing for de-hulled rapeseed, it is necessary to

improve the pushing ability of the feedstock delivering screw, strengthen the pressure and prolong pressing time.

2 Research about twin-screw cold pressing expeller

2.1 Design

New twin-screw expeller for cold pressing was designed to solve the above mentioned problems of single-screw expeller. And the design was as following.

(1) Twin-screw improved the pushing ability vastly, and the problem that de-hulled rapeseed was easy to slip in chamber was radically solved. Combination of joggling and non-joggling was designed in twin-screw expeller (Figure 1). Left and right worm of first screw segment joggled mutually. That was to say the screw bridge of one piece of screw inserted into the screw groove of another, and there was certain space around. Its ability to push the feeding was strong. The exterior diameter of both worms in the second screw was tangency, which was non-joggling. It not only has strong ability to push feedstock along axis, but also its structure fits to multiple-stage compression and relaxation and a thin layer of materials when pressing. This design had the characters of strong ability to push the feedstock in axis direction, and convenient regulation of the pressure in chamber and compression ratio of worm and pressing loop.

(2) Pressing cage and worm shaft were the heart of the expeller. However there were faults in single-screw expeller about its small ratio of length and diameter of pressing chamber and a little low total theoretical compressing ratio. To improve these faults, some changes in design principle and its structure had been made (Figure1). The total length of worm shaft was lengthened by the form of composition of multiple segment pressing screw and pressing loop. When many taper pressing loops were inserted into the space between two screws, multiple-stages compression and relaxation could be realized. Meantime, the ratio of length and diameter of pressing chamber and total theoretical compressing ratio were improved. In the main pressing segment, with the root diameter of pressing screw increasing gradually along the axis in vertical direction, the depth from the top of pressing screw teeth to the root reduced gradually. So a thin layer of materials when pressing was realized and the distance to exclude oil was shortened, which benefited improving the oil extraction rate.

(3) The two worm shafts circumvolved in different directions. The circumvolving direction was anti-direction from inside to outside which was decided by power beared and the oil excluding manner. When working, suspending worm shafts circumvolve centered automatically, which was also required when no-load.

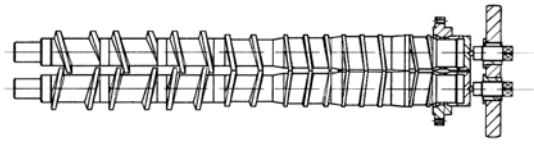
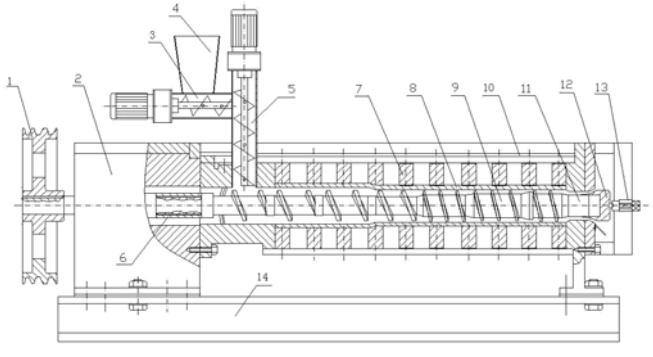


Fig.1 Sketch of twin-screw expeller with combination of joggling and non-joggling

2.2 Equipment structure

The module of twin-screw expeller was designed and manufactured according to the design mentioned above. Laboratory test of cold press for de-hulled rapeseed kernel was carried out and gained elementary success. Based on this, the equipment was improved and modified mainly about its delivering part, pressing chamber and pressure structure passing part. SSYZ50 twin-screw expeller was designed and manufactured successfully. The simply structure diagram was shown as figure 2.



1 Strap tray 2 Gear case 3 Level screw conveyor 4 Hopper 5 Vertical screw conveyor 6 Jointer 7 Pressing plate 8 Bar withstanding device 13 Accommodated mandril 14 Framework 9 Worm shaft 10 Pull rod 11 Outlet tray 12 Cake

Fig.2 Structural diagram of SSYZ50 twin-screw expeller

The main technical parameters of SSYZ50 twin-screw expeller were shown in table 1.

Table 1 Specifications of SSYZ50 twin-screw expeller

Item	Value
Total length of worm shaft (mm)	1800.0
Inner diameter of first stair pressing cage (mm)	160.5
Exterior diameter of first stair worm (mm)	154.0
Inner diameter of second stair pressing cage (mm)	140.5
Exterior diameter of second stair worm (mm)	136.0
Rotate speed of worm shaft (r/min)	10~18
Compressing ratio	23.0:1
Ratio of length and diameter	11.5:1
Press time (s)	180.0
Yield (T/d)	40~50
Power of principal electromotor (KW)	37~55
Externality dimension (length × width × height)mm	3900 × 2500 × 1800

2.3 Characteristics of this equipment

Compared with the traditional single-screw expeller, SSYZ50 twin-screw expeller had characteristics as following:

(1) Two screws delivered pressing oil in different directions in the same pressing cage, which was special structure. In the first segment of pressing chamber, left and right screws joggled mutually, and the feedstock between two screw grooves was half obturated. After feedstock particles entered pressing cage, they got the friction between screw thread and inner surface of pressing cage and friction among themselves. The moving state of feeding particles could be analyzed as circumvolving movement with axis and movement in axis direction. The main movement was circumvolving movement with axis, because the feedstock fraction was low. When feedstock moved in different directions to the joggling place, it got the function of press and friction. The circumvolving movement with the axis was destroyed, and the movement in axis direction increased obviously. So pushing function in axis direction with strong force came out. Screw groove was divided into small separated chambers with C shape in the joggling place, so feedstock was pushed ahead by the screw along the C chambers, and it was pressed lightly. During the second segment in the pressing chamber, exterior diameter of left and right screws was in tangency, and the two screw grooves communicated. Feedstock mainly went ahead with the screw, however, the function of mutual press and friction was also produced in tangency place. So the problems such as weak friction among de-hulled rapeseed kernels and feedstock r were ultimately solved.

(2) As showed in table 1, the design of twin-screw expeller provided high compressing ratio which could reach 23.0 theoretically, strong pressure in vertical direction which gave feedstock huge chemical pressure, high length and diameter ratio that reached 11.5:1, and prolonged the press time to 180s which made pressing drastically. Meanwhile thin layer of materials when pressing in long distance was realized and the distance of oil flow was shortened, which provided abundant time to press oil at high pressure. Actually the time to press oil at high pressure could reach more than 80s, so the yield ratio of pressing oil was improved. However, present ZX10 and ZX18 single-screw expeller had the ratios of length and diameter of press chamber of 6.3 and 6.72, respectively. The later had the theoretically compressing ratio 13.2, pressing time of 150s (Zhu & Shi, 1991)

3 The production and utilization state of twin-screw expeller

In July of 2004, SSYZ50 twin-screw expeller was successfully used in the new producing line of de-hulling, cold pressing, and expansion which was installed by Zhongpai Food and Oil Co. Ltd. in Wuhan, China. The production and utilization was as following.

The material of rapeseed was cleaned and dried, with water content about 8%. YTTP75 dehuller, which was designed and manufactured by Oil Crops Research Institute of Chinese Academy of Agricultural Sciences, was used to dehull and separate hull and kernel (Dehulling ratio was more than 95%). Rapeseed kernel with rate of 3-6% hull was gained. Rapeseed kernel was put into twin-screw expeller for cold pressing.

Table 2 The application results of twin-screw expeller for cold pressing of de-hulled rapeseed

Serial number	Water content of rapeseed kennerl(%)	Residual oil content of cold–pressed cake (%,dry matter basis)	Water content of cold–pressed cake(%)	Temperature of excluding cake(°C)
1	7.9	15.08	5.7	51
2	6.1	14.72	4.6	58
3	8.5	15.60	5.9	56
4	6.4	14.35	4.8	48
5	7.1	16.35	5.3	63
Average	7.2	15.22	5.3	55

Results were shown in table 2. Residual oil content of cold pressed cake was about 15%, which corresponded to residual oil content of pre-pressed cake. Better oil extraction rate was gained. Water content in cold pressed cake was 4-6%, which

descended by 1-2% compared with material of de-hulled rapeseed. Cold pressed cake came out at the temperature of about 55°C that was lower than 70°C, which fulfilled the requirement of cold pressing process. In the producing process, the equipment worked well, and the properties of the machine were stable. Disposal ability amounted to 45t/d.

The quality of cold pressing oil was tested by Quality Inspection and Test Center for Oilseeds and Products, Ministry of Agriculture, PRC. Results showed that some quality indexes such as colour and lustre, acidity were better than crude oil from pre-pressing process (Table 3). Its quality is better than the standard of the fourth grade rapeseed pressing Oil, almost as the standard of the third grade rapeseed pressing oil (Chinese national standard, GB1536-2004). Following refining process could be reduced. Contact with organic chemical reagent was avoided, so natural green production was gained.

Table 3 The quality of cold pressing double-low rapeseed oil

Index	The third grade rapeseed pressing oil	Cold pressing rapeseed oil
Colour and luster (Trough thickness 25.4mm)	≤Y35 R4.0	Y35 R4.0
Odor and taste	Having the inherent flavor and taste of rapeseed oil, no peculiar smell	Having the inherent flavor and taste of rapeseed oil, no peculiar smell
Acidity(mg KOH·g-1)	≤1.0	1.1
Moisture content and volatile materials(%)	≤0.10	0.04
Infusbility impurity(%)	≤0.05	0.01
Impurity(%)	≤0.10	0.01
Peroxide value(mmol/kg)	≤6.0	3.2
Heating test(280°C)	The colour allow to be deepen and not to be darken. Trace matter allow to be separate out.	The colour become deepen. No matter is separate out.
Soap content(%)	≤0.03	0.01

4 Conclusions

(1) In order to realize cold pressing of de-hulled double-low rapeseed, a twin-screw expeller with a principle of combination of joggling and non-joggling was designed. In the expeller, feedstock could be cleared away automatically, so the problem that de-hulled rapeseed feedstock was difficult to be delivered was solved. In the pressing chamber, multiple-stage compression and relaxation and a thin layer of materials when pressing were applied. The total theoretical compressing ratio of the twin-screw expeller and ratio of length and diameter of pressing chamber were improved obviously. Pressing time was prolonged. SSYZ50 twin-screw expeller was designed according these theories. The total theoretical compressing ratio of the twin-screw expeller reached 23.0, and the ratio of length and diameter of pressing chamber reached 11.5. Meanwhile pressing time prolonged to 180s.

(2) SSYZ50 twin-screw expeller used in oil processing corporations successfully. Results revealed that oil content of cold pressing cake from de-hulled rapeseed was about15%. Better yield ratio of cold pressing oil was gained which approached the standard of the third grade rapeseed pressing oil (Chinese national standard, GB1536-2004), and could be treated as natural green food. Cold pressed cake came out at the temperature that was lower than 70°C, which fulfilled the requirement of cold pressing process.

References

Elizabeth M.Prior, Vivekenand S.Vadke,Frank W.Sosulski. Effect of heat treatments on Canola Press Oils[J]. *J. Am. Oil Chem.Soc.,*1991, 68(6):401-406.

Huang F H. High profit processing and multiple value-added techniques of canola. *China Oils and Fats,* 2002,27(6):9-11.

Huang F H, Zhou L X, Li W L, Wang J W, Lu S G, Cheng X Y. Study on dry dehulling technology of rapeseed. *China Oils and Fats,* 2000,25(6):48-49.

Li S L, Hu J H, Liu X F, Lin G Y. Key technolog ies for cold pressing of de-hulled double- low rapeseed kernel for o il extraction. *Transact ions of the CSAE,*,2004,20(6):181-185.

Ragnar Ohlson. Modern processing of rapeseed[J]. *J. Am. Oil Chem. Soc.*, 1992,69(3):195-198.

Rasehorn H J, Deicke H D, Xin Y M. Theory and praxis of decortication and cold pressing of rapeseed. *China Oils and Fats,* 2000,25(6):50-54.

Xin Y N. Idea and application area of cold press of oilseed. *China Oils and Fats,*,2005,30(2):20-22.

Zhang L. Research and developmen t of equipmen t for peel ing rapeseed and separating hull and kernel. *Transact ions of the CSAE,*,2004,20(1):140-143.

Zheng J C. Discuss on oil extraction technics for high quality rapeseed.. *China Oils and Fats,*,2001,26(5):38-39.

Zhu D P, Shi T Z. Oil extraction technics. Zhengzhou: Henan Science and Technology Press,1991,143-157.

Production of selenium-enriched rapeseed peptides

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Abstract

Research has been carried out on extracting Selenium-enriched rapeseed protein isolate (RPI). Based on single factor experiment, the extraction conditions had been optimized by orthogonal analysis. The optimum conditions for phytic acid and glucosinolate extraction are that the Selenium-enriched defatted rapeseed meal is extracted 3 times with a solid to solution ratio of 1:10 at 45°C for 60 min each at a pH of 4.0. The optimum extraction conditions for Selenium-enriched rapeseed protein isolate are that the meal is extracted 3 times with a solid to solution ratio of 1:12 at 50°C for 35 min each under pH12.0.

Enzyme hydrolysis for Selenium-enriched rapeseed protein isolate has been carried out. An alkaline enzyme, alcalase, was selected to hydrolyze the Selenium-enriched rapeseed protein isolate. The substrate (having a concentration of 3%) was digested with alcalase (180000U/g) for 7 hours at 50°C and a pH of 8.0. Under this condition, 32.51% of the substrate is hydrolyzed. The nitrogen recovery rate is 91.28%. The crude production is then decolorized with active carbon and desalinized, concentrated and lyophilized. Molecular weight of Selenium-enriched rapeseed peptide is lower 1500 Dalton.

Key words: Selenium-enriched rapeseed protein isolates,enzyme hydrolysis,Selenium-enriched rapeseed peptides

Introduction

Selenium is a trace element which can be used to cure many disease such as cancer, cardiovascular and eye disease. It is highly profitable and profoundly remarkable to develop selenium-enriched products. Rapeseeds absorb and enrich inorganic-Se from Se-enriched soil and transform to Se-Met and Se-Cys. Rapeseeds can accumulate as high as 10ppm and are good organic-Se resource.

In this research, we optimized the conditions for preparation of Se-enriched rapeseed protein isolate from defatted rapeseed meal and then optimized the conditions for preparation of peptide using enzyme hydrolysis.

Material and Methods

Raw material

Selenium-enriched rapeseed from En-shi city of Hubei province

Table 1 Main ingredients of stuff

ingredient	rapeseed	husked rapeseed	defatted meal
crude protein (N*6.25, dry basis,%)	25.19	28.57	39.04
ash content (%)	4.4	3.4	5.9
crude fat content (%)	37.53	42.39	0.48
moisture content (%)	7.91	6.30	8.51
tannin content (%)	2.52	2.71	3.62
phytic acid content (%)	2.38	2.72	4.02
glucosinolate (mg/g)	14.01	16.36	25.11
selenium (ppm)	2.21	2.53	3.30

Experiment

Phytic acid and glucosinolate were extracted from Selenium-enriched rapeseed defatted meal in the condition of pH5.0. The supernate was got off by centrifugating and the precipitate was the low-phytic acid rapeseed meal in which Selenium-enriched rapeseed protein isolate was prepared. The water was added in the meal in a certain proportion then adjusted pH to extract the protein effectively. After the mixed liquid was centrifugated, the above fluid was collected and settled by adding acid to pH4.0. The sediment is Selenium-enriched rapeseed protein isolate.

Selenium-enriched rapeseed protein peptide was prepared from protein isolate. Before hydrolyzing, there is a pretreatment on protein of dissolving in water, heating up on above 70°C and then 30minutes later, cooling to room temperature, adjusting pH, adding enzyme liquid, hydrolyzing several hours on constant temperature, heating at 80°C to destroy the enzyme activity, centrifugating and collecting the clear fluid, that is protein peptide liquid.

The effect factors of enzyme hydrolyzing are the choice of enzyme, the dose of addition, the substrate concentration and the time. First, according to the different effect of proteolytic enzyme A, B, C and D, one of was choiced to hydroze the

Selenium-enriched rapeseed protein isolate. Then the study of the other 3 factors carried on. In the single factor experiment, every factor has five levels, such as the dose of enzyme addition (3×10^4U/g, 6×10^4U/g, 9×10^4U/g, 12×10^4U/g, 15×10^4U/g), the substrate concentration (1%, 3%, 5%, 7%, 9%), the enzyme hydrolysis time (4h, 5h, 6h, 7h, 8h). When one factor levels are changed, the others are changeless. Which level of one factor was choiced lay on the degree of hydrolysis (DH) and the recovery rate of nitrogen. Based on the single factor experiment, the hydrolysis conditions would be optimized by orthogonal analysis. The method for determination of DH is formaldehyde titrating rapidly to free amino nitrogen, and the recovery rate of nitrogen is Kjeltec nitrogen analyzer (HE Zhao-fan & ZHANG Di-qing, 1997; WANG Zhao-ci, 2000).

Results and Discussion

In this experiment, the condition of extracting phytic acid and glucosinolate had been confirmed. That was pH 4.0, solid to solution ratio 1:10, temperature 45°C, 3 times and 60min every time. The condition of preparing protein isolate was pH12.0, solid to solution ratio 1:12, temperature 50°C, 3 times and 35min every time. Under the technics, the yield of Selenium-enriched rapeseed protein isolate was 26% and protein content was 83.37%.

Table 2 the main target of Selenium-enriched rapeseed protein isolate

ingredient	content
protein (N*6.25, dry basis,%)	83.37
crude fat content (%)	0.61
moisture content (%)	6.34
tannin content (%)	1.41
phytic acid content (%)	0.5
glucosinolate (mg/g)	0
selenium (ppm)	5.87

Selenium-enriched rapeseed protein isolate was a middle product, it had high protein content, low fat content, and it was pretreatment to have a very low anti-nutrition content, such as phytic acid, glucosinolate and tannin etc. because selenium element is integrated some amino acids, it almost had no lost and its content had high enrichment. This is a very good base for peptide preparation.

In a following part of this single factor experiment, first several kinds of enzyme were operated to choice a better efficiency to increase DH and the recovery rate of nitrogen, showing as the figure 1. Then as show below figure 2 to figure 4, enzyme addition, concentration of substrate and enzymehydrolysis time the 3 single factors were carried one by one to choice the best level. The best level as a midpoint, 3 levels every factor were done orthogonal analysis experiment as below table 2.

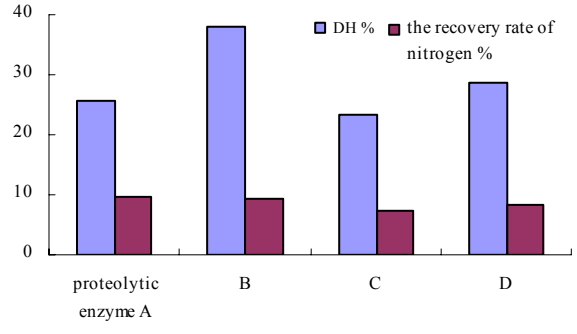

Fig.1 Effect of different enzymes on DH and the recovery rate of nitrogen solid to solution ratio1:20, the dose of enzyme3×10^4U/g, 50°C, 4h

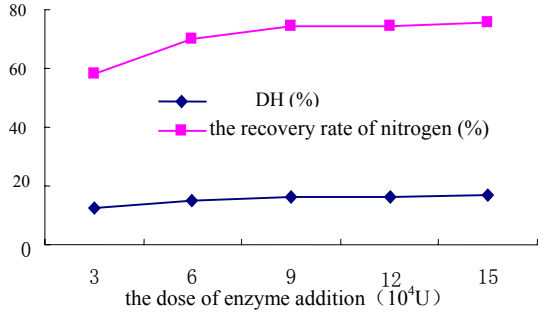

Fig.2 Effect of enzyme addition on DH and the recovery rate of nitrogen solid to solution ratio1:20, pH=8, 50°C, 4h

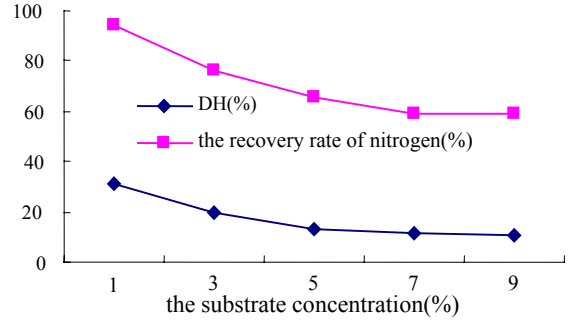

Fig.3 Effect of concentration of substrate on DH and the recovery rate of nitrogen
pH=8, the dose of enzyme addition9×10^4U/g, 50°C, 4h

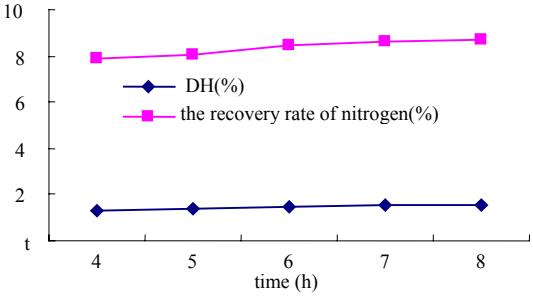

Fig.4 Effect of enzymehydrolysis time on DH and the recovery rate of nitrogen
pH=8, the dose of enzyme addition9×10^4U/g, 50°C

Table 2 result of orthogonal experiment

number	A dose of enzyme addition (10^4U/g)	B substrate concentration (%)	C time (h)	DH (%)	recovery rate of nitrogrn (%)
1	12	3	5	29.82	88.61
2	15	3	6	30.11	90.37
3	18	3	7	32.09	91.93
4	12	5	6	26.24	84.65
5	15	5	7	27.43	85.59
6	18	5	5	27.77	86.82
7	12	7	7	25.13	82.80
8	15	7	5	25.65	86.18
9	18	7	6	26.60	84.24
k1	27.063	30.673	27.747		
k2	27.730	27.147	27.650		
k3	28.820	25.793	28.217		
k1′	85.353	90.303	87.203		
k2′	87.380	85.687	86.420		
k3′	87.663	84.407	86.773		
R	1.757	4.880	0.567		
R′	2.310	5.896	0.783		

In the single factor of enzyme choice experiment, the proteolytic enzyme B had a higher DH and recovery rate of nitrogen than proteolytic enzymeA, C and D. So we should select the enzyme B to hydrolyze rapeseed protein for peptide. As figure2 show, along with the addition of proteolytic enzyme, the DH and nitrogen recovery rate were increased gradually. When the dose of enzyme was over 9×10^4U/g, the increase was a little slow, so we can select the dose 9×10^4U/g. From the figure 3, the DH and nitrogen recovery rate were declined obviously along with the increase of substrate concentration. If the addition of water is too high, the dose of protein will be too low correspondingly. Considering the cost is enhancive, so we can select the substrate concentration 3%. As the figure 4 show, along with the addition of hydrolysis time, the DH and nitrogen recovery rate were increased gradually. When the time was over 7h, the increase was a little slow, so we can select 7h as the best hydrolysis time.

From the table2, of the 3 effect factors, the effect of the substrate concentration is largest on rapeseed protein isolate hydrolysis, the addition dose of enzyme is taken second place, and the hydrolysis time is least. According to maximum variance analysis, the effect is B>A>C. According to the variance analysis of DH and the recovery rate of nitrogen, the remarkable effect factor is the substrate concentration. So the single factor tests and orthogonal experiments had been applied to optimize condition of enzymolysis as follows: pH8.0, substrate concentration is 3%, the time of enzymehydrolysis is 7 hours, temperature 50°C, and the dose of enzyme B is 18×10^4 U/g. Under the optimized condition, the drgree of hydrolysis (DH) is 32.51%, the recovery rate of nitrogen is 91.28%.

Refining

The hydrolysis product of Selenium-enriched rapeseed protein was yellowy fluid. It was dealt with pulverous active carbon to decolorize. The discoloring condition was pH3.0, the dose of active carbon 1%, heating temperature 70°C, the time 1h. Then filtrate when being hot, it was colorless. Whereafter the peptide liquid was desalinated with a dialysis bag which can intercept molecule weight 100. Finally the peptide was concentrated and was dried to be finished product. The ingredients of finished product are following:

Table 3 The main target of Selenium-enriched rapeseed protein peptides

ingredient	content
protein(N*6.25, dry basis,%)	87.11
crude fat content (%)	0.39
moisture content (%)	8.91
ash content (%)	4.79
NSI (%)	95.45
tannin content (%)	1.28
phytic acid content (%)	0.23
glucosinolate content (mg/g)	0
selenium (ppm)	6.02
solubility in TCA (%)	95.11

Conclusions

Selenium-enriched rapeseed husked and defatted meal was as a raw material to prepare rapeseed protein isolate that was pretreated to reduce its phytic acid and glucosinolate content. Rapeseed protein isolate was a middle product to prepare rapeseed peptide by enzyme hydrolysis. The composing ingredient of this peptide production is: protein content 87.11%, NSI 95.45%, solubility in TCA 95.11%, moisture content 8.91%, tannin content 1.28%, phytic acid content 0.23%, glucosinolate content 0, selenium content 6.02ppm. After determining, molecular weight distributing of peptide production is well, and it

almost all is small molecular peptide. The Selenium-enriched rapeseed protein peptide has lower 1500D average molecular weight. The rate of 926D is 78.28%, 675D is 18.89%, 233D is 2.9%.

References

Bigelow C C, (1967). On the Average Hydrophobicity of Proteins and the Relation Between it and Protein Structure, J Theoret Biol, **16**, 187-211
HE Zhao-fan, ZHANG Di-qing, (1997). Chemistry and determination technique of health food product, Bei-jing, China.
John, A.M., (1984). Selenium and the transplantable tumor, Agric. Food Chem., **32**, 437-438
LI Qiu-yue. (1987). General utilization of rapeseeds, Bei-jing, China.
Netto FM, Galeazzi AM. (1998). Production and Characterization of Enzymatic Hydrolysate From Soy Protein Isolate, Food Sci Technol-Leb, **31**, 624-631
Passuater, R.A., (1980). Selenium as Food and medicine, Keats publishing.
WANG Zhao-ci, (2000). Quality analysis of grain and oil product, Bei-jing, China.

Enzymatic transesterification of *Brassica juncea* seed oil for production of neutraceuticals

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Abstract

The importance of polyunsaturated fatty acids (PUFA) in human nutrition and disease prevention has long been recognized. Both omega-3 and omega-6 PUFA are precursors of eicosanoids, which are involved in many important biological processes in the human body. GLA (gamma linolenic acid) is important for the prevention and treatment of skin disease, premenstrual syndrome, diabetes, inflammatory and autoimmune disorders and cancer. Mustard (*Brassica juncea*) oil could produce neutraceuticals under enzymatic interesterification with short chain fatty acids (C_8-C_{10}) and other important fatty acids. An immobilized lipase from *Thermomyces lanuginosus* (TL IM) could be employed to mediate the continuous transesterification of mustard oil with other unsaturated oils in a packed bed reactor operating at 60-65^{0}C. Introduction of EPA (Eicosapantanoic acid), DHA (Docosahexaenoic acid) and GLA (Gamma linolenic acid) in mustard oil could contribute to the removal of erucic acid and also could be utilized for DAG (Di-acyl glycerol) by taking glycerol and 2-position fatty acids esterification in presence of ethyl alcohol or isopropanol. These products could be complex mixtures of diacylglycerol (DAG) species with good amount of unsaturation and essential fatty acids in appropriate ratio, whose composition depend on reaction conditions.

Key words: Lipase, interesterification, PUFA, DAG, GLA, DHA, EPA, neutraceutical, structured lipids

Introduction

Structured lipids(SLs) are tailor-made fats and oils with improved nutritional or physical properties because of modifications to incorporate new fatty acids or to change the position of existing fatty acids on the glycerol backbone. More recently, SLs were designed to provide simultaneous delivery of beneficial long chain fatty acids (LCFAs) at a slower rate and medium chain fatty acids (MCFAs) at a quicker rate (Babayan 1987; Akoh 1998). SL synthesis yields novel triacylglycerol (TAG) molecules (Akoh 1998). SLs may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes, and for targeting specific diseases and metabolic conditions (Lee and Akoh, 1998). Improvements or changes in the physical and/or chemical characteristics of a TAG can also be achieved when SLs are synthesized

The component fatty acids and their position in the triacylglycerol (TAG) molecule determine the functional and physical properties, the metabolic fate, and the health benefits of an SL. MCFAs are preferentially transported via the portal vein to the liver, because of their smaller size and greater solubility compared to LCFAs (Bell and others 1991; Straarup and Hoy 2000). Therefore, it appears that MCFAs are most useful in a structured lipid that combines their inherent mobility, solubility, and ease of metabolism with more healthful polyunsaturated fatty acids (Cater and others 1997; Akoh 1998). LCFAs are absorbed and metabolized more slowly than either medium or short chain fatty acids; much of the LCFAs may be lost as calcium-fatty acid soap in the feces (Broun and others 1999). Eicosapentaenoic acid, 20:5n-3 (EPA), and docosahexaenoic acid, 22:6n-3 (DHA), found in fish oil, are other n-3 polyunsaturated fatty acids (PUFAs) of interest in SL production.

Therefore, it is evident from the above statements that development of structured lipids is very important for their application in functional food, neutrceuticals, low calorie fats etc. In the present study mustard oil was selected because it contains good amount of n-3 and n-6 fatty acids and was interesterified with EPA and DHA. This process makes mustard oil as a neutraceutical oil for various functional purposes.

Materials and methods

Mustard oil used in experimentation was purchased from local market, Lipase enzyme (Lipozyme TL 1M) was obtained from Novozyme, EPA and DHA was purchased from Merck in the form of Maxepa capsules.

EPA and DHA was first esterified with methanolic potassium hydroxide in organic solvent medium, ester layer was separated and added to mustard oil. The mixture was added with 10% lipase enzyme and was incubated at 60°C for different time periods viz 4, 6, 8 and 12 hours with constant shaking on shaker water bath system. Fatty acid composition of normal mustard oil and the Maxepa from Merck was also undertaken

After the interesterification process for different time periods was completed the mixture was then centrifuged at 10000 RPM for 10 minutes. After centrifugation the enzyme layer oil oil-ester layer was separated. The oil-ester layer was then spotted on silica gel plate with 10 replicates for each treatment. The spotted silica gel plate was then allowed to run in hexane-diethylether (70:30) mobile phase. These plates were allowed to run upto 10cm from the spot. After taking out the plates the lower 0.5cm from spotted point was scratched and dissolved in 10ml heptane and esterified with methanolic

potassium hydroxide for detailed GLC analysis.

Results

The fatty acid composition of mustard oil and Maxepa capsule was analyzed after converting them into methanolic esters. In Table1 the detailed fatty acid composition shows that mustard oil employed in this study contains 48% erucic acid and Maxepa contains 18% EPA and 12% DHA in addition to other fatty acids.

Table 1: GLC profile of major fatty acids of mustard oil and Maxepa

S.No.	Fatty acid composition	Mustard Oil	Maxepa
1.	Oleic	10.39%	16.27%
2.	Linoleic	15.79%	2.66%
3.	Linolenic	12.38%	1.16%
4.	Erucic	48.01%	0.00%
5.	EPA	0.00%	18.05%
6.	DHA	0.00%	11.82%

In our study we have observed that as the time period for enzymatic hydrolysis increases the interesterification reaction decreases after a certain time, which was 6 hours. In Table 2 we have observed that among the four treatments of different time periods the maximum incorporation of EPA and DHA is in

Table 2: GLC profile of major fatty acids of interesterified oil at different reaction periods

S.No.	Fatty acid composition	4hr reaction	6hr reaction	8hr reaction	12hr reaction
1.	Oleic acid	30.35%	34.00%	33.94%	28.00%
2.	Linoleic acid	13.48%	15.45%	18.10%	15.92%
3.	Linolenic acid	6.58%	8.31%	15.23%	8.73%
4.	Eruic acid	0.40%	0.26%	0.10%	0.00%
5.	EPA	27.00%	18.83%	16.00%	27.31%
6.	DHA	7.57%	4.76%	3.30%	4.30%

All fatty acid composition is the average of ten hydrolysis replicates

4 hours reaction but at the same time the erucic acid left in the oil is 0.4% as compared to 6 hours reaction in which the erucic acid left in the interesterified oil is 0.26% only and also the maximum interesterification percentage was observed in this time period.

As the time for reaction increased from 6 to 12 hours the reaction reached to equilibrium and then showed a downtrend. Lipase-catalyzed reactions were a combination of esterification and hydrolysis (reverse reaction) reactions. After reaching equilibrium these reactions showed stagnation and then a downtrend. Structured lipids produced with lipases in organic solvent, where substrates are soluble and hydrolysis can be minimized depending upon the objective for structured lipids. It was understood during the different reactions performed that the type of organic solvent employed dramatically affected the reaction kinetics and catalytic efficiency of an enzyme. The extent to which the solvent affected the activity or stability of the enzyme and the effect of the solvent on the equilibrium position of the desired reaction must both be considered when choosing a solvent for biocatalysis.

Discussion

In our study it is evident that mustard oil can be designed to a 'Neutraceutical oil' with the incorporation of EPA and DHA with lipase catalyzed interesterification. The removal of erucic acid and incorporation of essential fatty acids for the purpose of making it fit for various functional foods. From the study it is also evident that enzymatic interesterification is time and temperature sensitive therefore a proper time period analysis is very important. Also, lipases occur widely in nature and are active at the oil-water interface in heterogeneous reaction systems. Lipase catalyzed interesterification reactions offer the advantage of greater control over the positional distribution of fatty acids in the final product, due to lipases' fatty acid selectivity and regiospecificity. Lipases hydrolyze triacylglycerol (TAGs) to monoacylglycerols, diacylglycerols (DAGs), free fatty acids (FFA), and glycerol. In addition to the ester-interchange reaction, lipases can also catalyze direct esterification, acidolysis, and alcoholysis reactions (Lee and Akoh, 1998). As is evident from the 6 hours reaction onwards where it is clear that the reaction time period increases the interesterification rate and then shows a downtrend. In present study at 60°C the interesterification process was found optimum for a period of 6 hours. Most lipases are optimally active between 30 and 40°C (Shahani 1975). As the temperature increases, enzyme molecules unfold by destruction of bonds, such as sulfide bridges, and may lead to hydrolysis of peptide bonds and deamidation of asparagines and glutamine residues. However, these processes can be avoided in a water-free environment. Immobilization of enzymes also results in greater thermo stability. Additionally, genetically engineered lipases are now available for the synthesis of SLs. It is hoped that the use of biotechnology will reduce the cost of lipases, making the enzymatic route to SLs economically viable. Other factors affecting enzymatic activity and product yield include pH, substrate molar ratio, enzyme activity and load, incubation time, specificity of enzyme to substrate type and chain length, and regiospecificity (Akoh, 1998). Two of the most attractive reasons for choosing enzymatic over chemically catalyzed reactions for SL production are the energy saved and minimization of thermal degradation.

Conclusion

The study shows that mustard oil although contains essential fatty acids in well balance but still it can further be modified into Neutraceutical oil and for particular functional food purposes. The incorporation of EPA and DHA enhances its nutritional and functional value manifold. Although much remains unstudied in the field of SLs, but still the properties of a SLs for reduction in calorific value of foods that normally contain high amounts of fat, or the medicinal properties of rapidly absorbing TAGs composed of medium chain fatty acids (MCFAs) and poly-unstaurated fatty acids (PUFAs), structured lipids definitely provide attributes that consumers will find valuable. Therefore, it is important that further research be conducted that will allow for better understanding and more control over the various esterification processes and reduction in costs associated with large-scale production of SLs.

References

Akoh C.C. (1998). Structured lipids. In: Akoh CC and Min DB, editors. Food lipids chemistry, nutrition, and biotechnology. New York: Marcel Dekker. P 699-727.

Babayan V.K. (1987). Medium chain triglycerides and structured lipids. Lipids **22(6):**417-420.

Bell S.J., Macioli E.A., Bistrian B.R., Babayan V.K., Blackburn G.L. (1991). Alternative lipid sources for enteral and parenteral nutrition: long- and medium-chain triglycerides, structured triglycerides, and fish oils. J Am Dietetic Assoc **91(1)**:74-78.

Broun P., Gettner S., Somerville C. (1999). Genetic engineering of plant lipids. Annu Rev Nutr **19(1)**:197-216.

Cater N.B., Howard J.H., Denke M.A. (1997). Comparison of the effects of medium-chain triacylglycerols,

Lee K.T., Akoh C.C. (1998). Structured lipids: synthesis and applications. Food Rev Int **14(1):**17-34.

Shahani K.M. (1975). Lipases and esterases. In: Reed G, editor. Enzymes in food processing.2nd ed. New York: Academic Press. P 182-221.

Straarup E.M., Hoy C.E. (2000). Structured lipids improve fat absorption in normal and malabsorbing rats. J Nutr **130(11):**2802-2808.

Tocopherols, tocotrienols and a new radical scavenging substance in raw and processed rapeseed and rapeseed oil

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Abstract

The content of tocopherols (alpha, gamma, delta), alpha-tocotrienol and plastochromanol-8 was determined both in raw and processed rapeseed as well as in different rapeseed oils after saponification and hexane extraction by normal-phase liquid chromatography using fluorescence detection. The results show that gamma-tocopherol represented 54% and alpha-tocopherol 32% of the total tocopherols. The content of plastochromanol-8 accounted to 12% of the total tocopherols. The alpha-tocotrienol content was below 1%, which was separated with a high resolution. Applying a modified method of tocopherol analysis by decreasing the isopropanol fraction in the solvent a strong phenolic antioxidant was found in the crude rapeseed oil. The compound was identified as 4-vinylsyringol also referred to as canolol, a decarboxylation product of sinapinic acid. For the structural identification 4-vinylsyringol was isolated from crude rapeseed oil. 4-Vinylsyringol was chemically synthesized and compared with the natural compound. 4-Vinylsyringol was detected in cold pressed oil and in roasted rapeseed but not in fully refined oils. Interestingly, the 4-vinylsyringol content increased after roasting the rapeseed.

Key words: rapeseed oil, *Brassica napus*, tocopherols, tocotrienols, plastochromanol-8, 4-vinylsyringol, canolol

Introduction

Rapeseed and rapeseed oil are particularly rich in phenolic substances. The content of the substances is substantially higher than in soy bean, cotton, peanut, and sun flower oil (Kozlowka et al., 1990, Ciz et al., 2002). The concentration of the phenols is higher in raw rapeseed oil than in refined rapeseed oil. The loss of the phenols by the processing is also associated with a decrease of the antioxidative capacity and stability (Ciz et al., 2002). The majority of the phenols in rapeseed oil are non-polar substances like the tocopherols and tocotrienols. In the oil processing the most polar phenols remain in the rapeseed meal. In rapeseed oil 4-vinylsyringol is the predominating polar phenol. 4-Vinylsyringol is a stronger antioxidant than alpha-tocopherol, vitamin C, and beta-carotene as far as alkyl peroxyl radicals are concerned (Wakamatsu et al., in 2005). In other investigations anti-mutagenic and anti-inflammatory properties were also proved (Wakamatsu et al., 2005, Vuorela et al., 2005).

Material and Methods

2 g of the rapeseed oil were dissolved in 10 mL *n*-hexane and a volume of 20 µL was used for injection. Tocopherols, tocotrienols, plastochromanol-8, and 4-vinylcanolol were separated by a Shimadzu 10A series HPLC using a 250 mm x 4 mm, 5 µm Nucleosil-100 NH_2 column (Macherey-Nagel, Dueren, Germany), by isocratic elution with *n*-hexane/2-propanol (96:1.2 v/v) at a flow rate of 1.2 mL/min. The tocopherols, tocotrienols plastochromanol-8, and 4-vinylsyringol were detected using a RF 10AXL fluorescence detector (excitation wave length: 298 nm, emission wave length: 325 nm) and diode-array detector (275 nm), respectively. The rapeseed oil was analyzed before and after roasting in a microwave for 6 min (600 Watt). The rapeseed phenolics were extracted with *n*-hexan/ethylacetat (50:50 v/v) in a soxhlet extractor. The extract was evaporated to dryness in a rotavapor at 40°C. The residues were redissolved in *n*-hexan (10 mL) and 1 mL of this was diluted with the mobile phase to 10 mL. The parameters for the HPLC analysis were the same as for the oils.

Results

The results show that gamma-tocopherol represents 54% and alpha-tocopherol 32% of total tocopherols. The content of plastochromanol-8 accounted to 12% of the total tocopherols. The alpha-tocotrienol content was below 1%, wich was separated with a high resolution.

The alpha-tocopherol content was not affected by the oil processing. However, the alpha-tocotrienol, gamma-tocopherol and plastochromanol-8 content of cold pressed and crude oil was higher than that of refined oil (Tab. 1). Applying a modified method of tocopherol analysis by decreasing the isopropanol fraction in the solvent a strong phenolic antioxidant was found in the crude rapeseed oil. The compound was identified as 4-vinylsyringol, a decarboxylation product of sinapinic acid. For the structural identification 4-vinylsyringol was isolated from crude rapeseed oil. 4-Vinylsyringol was synthesized and compared with the natural compound. 4-Vinylsyringol was detected in crude oil, cold pressed oil and in roasted rapeseed but not in fully refined oils and steam stripped oil (Fig 1). Because 4-vinylsyringol was found in canola oil it is also referred to as canolol. The level of canolol in rapeseed was determined before and after roasting. The canolol content increased after roasting the rapeseed by approximately 120 times (Fig. 1 and 2).

Table 1. Tocopherol and tocotrienol content in the tested rapeseed oils [mg/100g]

Rapeseed oil	alpha-tocopherol	alpha-tocotrienol	gamma-tocopherol	delta- tocopherol	plastochromanol-8	total tocopherols
Cold pressed	29.7	1.2	45.1	1.8	10.1	87.9
Refined	28.4	< 0.2	40.0	1.6	7.0	77.0
Kernel oil	20.2	1.0	43.3	2.1	10.8	77.4
Washed	30.4	< 0.2	40.6	1.3	10.5	82.8
Crude	35.0	< 0.2	48.4	2.2	10.1	95.7

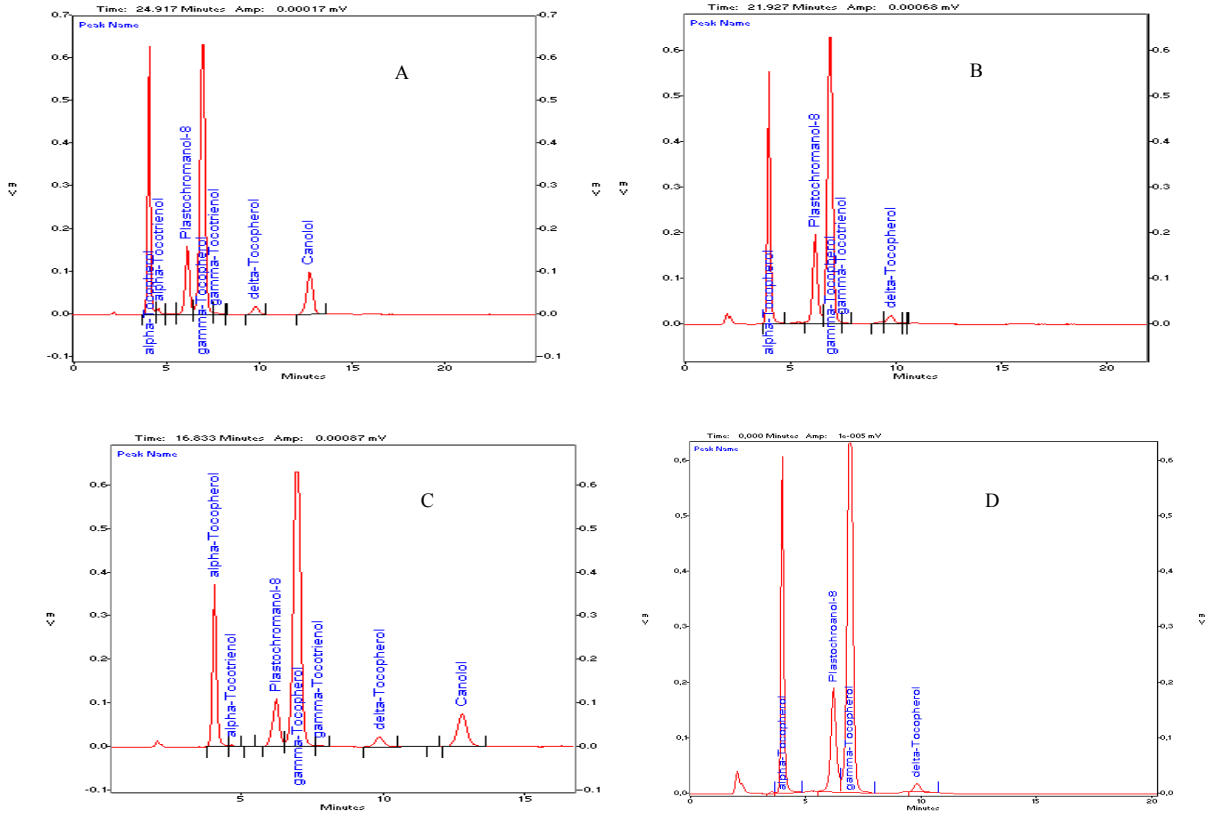

Fig. 1. HPLC chromatogram of different rapeseed oils samples; A: cold pressed rapeseed oil; B: refined rapeseed oil; C: kernel oil; D: washed rapeseed oil

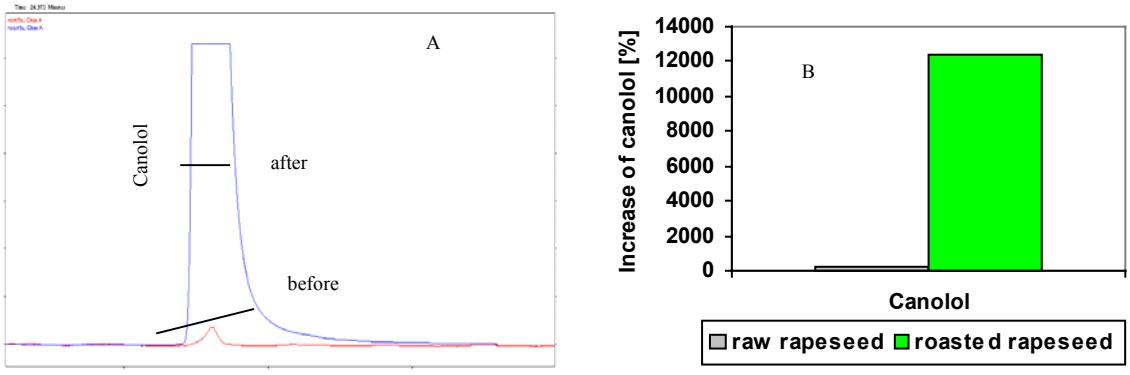

Fig. 2. A: HPLC chromatogram of rapeseed sample before and after roasting; B: Comparison between the increase of canolol before and after roasting

Conclusion

In conclusion, crude rapeseed oil comprises of a high content of antioxidants like tocopherols, tocotrienols, plastochromanol-8, and canolol. The present results suggest that the amount of the antioxidants particularly the content of canolol is reduced or completely removed after refining. Notable is the abundant increase of canolol after roasting rapeseed.

References

Ciz, M., Gorinstein, S., Lojk, A, Martin-Belloso, O., Leontowich, H. and Trakhtenberg, S. (2002): Comparative antioxidative properties of selected seed oils.

Free Radical Research, 36S1: 64-65
Koski, A., Pekkarine, S., Hopia, A., Wähälä, K. and Heinonen, M. (2003): Processing of rapeseed oil: Effect of sinapic acid derivative content and oxidative stability. European Food Research and Technology, 217: 110-114
Kuwahara, H., Kanazanwa, A., Wakasamatsu, D., Morimura, S. Kida, K., Akaike, T. and Mead, H. (2004): Antioxidative and antimutagenic activities of 4-vinyl-2,6-dimethoxyphenol (canolol) isolated from canola oil. Journal of Agriculture and Food Chemistry, 52: 4380-4387
Pekkarinen, S., Hopia, A.and Heinonen, M. (1998): Effect of processing on the stability of low erucic acid turnip rapeseed oil. Fett/Lipid 100:69-74
Vuorela, S., Meyer, A.S. and Heinonen, M. (2004): Impact of isolation method on the antioxidative activity of rapeseed meal phenolics. Journal of Agriculture and Food Chemistry, 52: 8202-8207
Vuorela, S., Kreander, K., Karonen, M. et al. (2005): Preclinical evaluation of rapeseed, raspberry, and pine bark phenolics for health related effects. Journal of Agriculture and Food Chemistry, 53: 5922-5931
Wakasamatsu D., Morimura, S., Tomohiro, S., Kida, K., Nakai, C. and Maeda, H. (2005): Isolation, identification, and structure of a potent alkyl-peroxyl radical scavenger in crude canola oil, canolol. Bioscience, Biotechnology, Biochemistry, 69: 1568-1574

Accumulation of pesticides residues in oil during the storage of rapeseed

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Abstract

Although pesticides residues are rarely present in crude rapeseed oils and are eliminated during the different steps of refining, it is important to know the origin of these contaminations to minimize the risks through good management practices and education. A study led between 2003 and 2005 showed that the main contaminants were organophosphate compounds (dichlorvos, chlorpyriphos-methyl, pyrimiphos-methyl, malathion) and pyrethrinoids (deltamethrin) which are insecticides traditionally used for cereal grain treatments during storage. The French regulation does not authorize these insecticides for the treatment of stored oilseeds which have however an affinity for liposoluble chemicals and can pick these up from storages which have previously been treated or used to store treated cereal grains.

We have measured the uptake of pesticides from treated cell walls by stored rapeseeds, using an experimental model : spraying of pyrimiphos-methyl (0,2 g of active material/m^2) on metal sheets covered by a single layer of rapeseeds or wheat after a drying period of 24h and measure of pyrimiphos-methyl residues in seeds and grains after 8 and 29 days. The pyrimiphos-methyl content in rapeseed was 0,028 mg/kg at the beginning of the experience, 15 mg/kg at J+8 and 15 mg/kg at J+29 (MRL 0,05 mg/kg). The pyrimiphos-methyl content in wheat was 0,062 mg/kg at the beginning of the experience, 15 mg/kg at J+8 and 17 mg/kg at J+29 (MRL 5 mg/kg). It was concluded that the transfer of pyrimiphos-methyl is fast (the values are equal at J+8 and J+29 and similar in rapeseed and wheat). Extrapolation studies in small storage silos (capacities 10 and 30 tons) are in progress for evaluating the average values of pesticides that can be really detected in seeds and grains.

Key words: pesticide residues, rapeseed, wheat, storage, pyrimiphos-methyl

Introduction

European consumers expect food safety and are particularly concerned about pesticide residues in food. Grains and seeds storage is a risky step because insecticides are used in a confined space where these molecules are not much damaged, unlike in a field. Insecticide treatments are applied on stored foodstuffs (cereals) in order to fight against pests : organophosphate compounds (dichlorvos, malathion, pyrimiphos-méthyl) or pyrethrinoids (deltamethrin). Such treatments are also applied on empty grain silos walls and on empty handling and storage equipment for the same reason. But, for oilseeds, no insecticide is allowed directly on seeds during storage, so MRLs (Maximum Regulatory Limits) for insecticides used in elevators are very low or do not exist on oilseeds. Some industrial specifications require no insecticide residues in cereals. Thus, foodstuffs, stored in elevators where are used insecticides, can be contaminated by contact with treated walls and equipment These insecticide residues in oilseeds can be a trade disagreement when MRLs are exceeded. The insecticide residues are removed in rapeseed oil after crushing and oil extraction because of the liposolubility. But refining steps eliminate theses residues, so that marketed refined oil is pesticide residue free and does not mean a risk for consumer health.

Studies have been led in Canada on insecticide residues transfer in stored rapeseed or cereals on various surfaces (plywood, galvanized steel surfaces, concrete) from 1976 to 1985. The molecules studied were organophosphorous (malathion, pyrimiphos-methyl, fenitrothion, bromophos, iodenphos) and pyrethrinoids (cypermethrin, fenvarelate, permethrin). Results showed that modification of each parameter (surface, seed, molecule) change residues levels obtained. Indeed, the uptake of insecticides is higher on plywood and galvanized steel surfaces than on concrete. Depending on the seed, uptake of insecticide from surface changes ; Watters (1982) showed that bromophos uptake is higher by rapeseed than wheat. The kind of molecule applied on surfaces, temperature and moisture can also modify insecticide residues concentrations in seeds. Insecticide residues movement through seeds layer is slight (White 1984) and malathion in stored barley is present no more than 2 cm from treated steel surfaces.

A survey led in France between 2003 and 2005 showed that the main contaminants in rapeseed were organophosphate compounds (dichlorvos, chlorpyriphos-methyl, pyrimiphos-methyl, malathion) and pyrethrinoids (deltamethrin), with low content (few hundreds of µg/kg found in rapeseed, versus few mg/kg when seeds are directly treated). Our hypothesis is that these seeds contaminations come from contact with treated surfaces, or contact with handling and storage system previously used by treated cereals. The aims of this study in 2005 were to measure this insecticide residues transfer in laboratory conditions and to compare with previous results obtained on cereals in order to extrapolate to small lots of stored seeds.

Materials and methods

Galvanized steel panels of 200 cm^2 were arranged in a random way on 10 m² polyethylen sheets. Each sheet was treated

with pyrimiphos-methyl at 0,2 g of active substance/m² (commercial product, Pirigrain 250) with a manual sprayer. After 24 hours drying at room temperature (about 20°C), we got the treated metal panels and put a single layer of rapeseed and wheat on them. Rapeseed and wheat come from organic agriculture.The same operation was led the day after. Two durations of contact between treated panels and seeds were studied : 8 days and 29 days. Temperature was registered during all the experiment with a thermometer probe within the seeds.

Analysis method for pyrimiphos-methyl residues in seeds was GC/IT/MS/MS.

Results

Table 1 - Pyrimiphos-methyl residues (mg/kg) in rapeseed and wheat stored on treated panels for two durations (mean of two repetitions)

	Initial concentration	8 days	29 days
Wheat	0,062	15	17,5
Rapeseed	0,028	17,5	14,5

Table 2 - Uptake rate* of pyrimiphos-methyl by wheat and rapeseed for two durations (mean of two repetitions)

	8 days	29 days
Wheat	17,7%	20,6%
Rapeseed	10,7%	8,9%

* Uptake rate = [(final pyrimiphos-methyl residue – initial concentration)* seed weight on the panel] / pyrimiphos-methyl quantity applied on the panel

Results show that pyrimiphos-methyl uptake by seeds (wheat and rapeseed) from treated metal surfaces exists and this uptake is quite the same for both kinds of seeds (table 1) : insecticide residues found for 8 days contact duration (mean of two repetitions) are 15 mg/kg in wheat and 17,5 mg/kg in rapeseed. For a contact during 29 days, values are similar between both kinds of seeds : 17,5 mg/kg in wheat et 14,5 mg/kg in rapeseed. The insecticide uptake by seeds from surface is rapid, since values after 8 days are quite the same than after 29 days of contact. The Maximum Regulatory Limits (MRLs) for pyrimiphos-methyl in wheat and rapeseed are exceeded : wheat MRL is 5 mg/kg, rapeseed MRL is 0,05 mg/kg. But these results are not a realistic situation because we only analyzed a single layer of seeds, so all the seeds were in contact with treated surface.

It is also interesting to study the uptake rates (table 2), which is the ratio between active substance quantity found in seeds and initial quantity applied on surface, a percentage of pyrimiphos-methyl uptaken by seeds. This rate is higher for wheat than rapeseed for 8 days contact duration : 17,7% versus 10,7%. For a 29 days contact duration, the results are the same : 20,6% for wheat and 8,9% for rapeseed. These differences coud be explained by the physical seed characteristics : seed shape, cuticule structure and insecticide migration ability within the seed.

Discussion

The National laboratory for stored seeds (NLSS) and Arvalis - Institut du Végétal (french technical institute for cereals and forage) led similar experiments 3 and 4 years before, with the same aim. During 2001, experiments at two scales :

On panels in laboratory (galvanized steel and concrete) with a single layer of wheat (same procedure than above). Tested substances : deltamethrin and pyrimiphos-methyl.

In small storage bin (10 tonnes) : treatment on walls when empty bin, and analysis of residues in wheat after two storage durations (1 week, 1 month). Tested substances : dichlorvos and pyrimiphos-methyl.

During 2002, Arvalis - Institut du Végétal made the same experiment than previous year but on taller bins : 30 tonnes, with two storage durations (1 month and 2 months). Tested substance : pyrimiphos-methyl.

Fo all these experiments, seeds were put in contact with surfaces or bins 24 hours after surface treatment.

Uptake rates were calculated in order to compare the results of the three experiments.

Table 3 Uptake rate of pyrimiphos-methyl by wheat in 10 tonnes and 30 tonnes bins for two durations (Arvalis 2001-2002)

10 tonnes bin	7 days	8,50%
	28 days	5,33%
30 tonnes bin	1 month	8,70%
	2 months	9,60%

Table 4 - Uptake rate of pyrimiphos-methyl by a single layer of wheat on treated panels for three durations (NLSS 2001)

8 days	**2,77%**
15 days	**7,86%**
30 days	**9,63%**

Results obtained by CETIOM in 2005 (table 2) confirm what was observed by NLSS in 2001 (table 4) : an increase of residues quantity in the time. Higher uptake rates obtained by CETIOM can be explained by temperature conditions when pyrimiphos-methyl was applied (20°C for CETIOM experiment and 25-26°C for NLSS experiment). With 25°C of ambient

temperature, more pyrimiphos-methyl could volatilize just after the treatment.

Table 5 Extrapolation of 2005 CETIOM results obtained on panels for rapeseed to small storage bins

Contact duration	Bin weight	Bin surface	PMM* treatment	PMM* quantity	Stored rapeseed weight	Uptake rate on panels	Calculated PMM* residues in rapeseed
days	tonnes	M²	g/m²	g	kg	%	mg/kg
8	10	25	0,2	5	9185	10,79	0,059
8	30	50	0,2	10	27555	10,79	0,039
29	10	25	0,2	5	9185	8,94	0,049
29	30	50	0,2	10	27555	8,94	0,032

PMM = pyrimiphos-methyl

Extrapolation of results obtained in laboratory (uptake rates measured) to storage bin of 10 tonnes show that MRL (0,050 mg/kg of PMM in rapeseed) is exceeded after 8 days. In 30 tonnes bins, the PMM residues are a little lower than MRL.

If other handling and storage system are covered with insecticide, risk of non-conformity exists.

Extrapolation from galvanized steel panels to farm scale storage bin is not really reliable. It should be confirmed by experiments in 10 and 30 tonnes bins.

Conclusion

According to calculations, the larger are elevators the higher is the ratio between volume of foodstuffs and treated surface. So that the risk of non-conformity is smaller by transfer of insecticide residues from treated walls. In big elevators of grain storage companies (more than 1000 tonnes), the risk to obtain more residues than MRL for rapeseed (0,05 mg/kg) is slight, contrary to small bins (less than 50 tonnes).

But in important elevators, insecticide uptakes by seeds can also occur at other steps : conveying belts, handling of oilseeds after treated cereals in the same circuits, bushels of forwarding, … So we cannot rule out either this risk for grain storage companies.

This study showed that uptake of insecticide residues by oilseeds from a treated metal surface exist and can lead to non-conformity for storage at farm scale. Other subjects should be studied : effect of duration between surfaces treatment and seed storage, effect of temperature and moisture.

Acknowledgments

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References

Mensah, G.W.K., Watters, F.L., Webste, G.R.B., 1979. Translocation of malathion, bromophos, and iodofenphos into stored grain from treated structural surfaces, Journal of economic entomology, vol.72, n°3, pp385-391.

Mensah, G.W.K., White, N.D.G.,1984. Laboratory evaluation of malathion-treated sawdust for control of stored-product insects in empty granaries and food warehouses, Journal of Economic Entomology vol. 77, n°1, pp202-206.

Watters, F.L., 1976. Persistance and uptake in wheat of malathion and bromophos applied on granary surfaces to control the red flour beetle, 69, n°3, pp353-356.

Watters, F.L., Nowick,i T.W., 1982. Uptake of bromophos by stored rapeseed, Journal of Economic Entomology, vol.75 n°2, pp261-264.

Watters, F.L, White, N.D.G., Coté, D., 1983. Effect of temperature on toxicity and persistence of three pyrethroid insecticides applied to fir plywood for the control of the red flour beetle, Journal of economic entomology, vol.76, n°1, pp11-16.

White, N.D.G., 1982. Effectiveness of malathion and pyrimiphos methyl applied to plywood and concrete to control *Prostephanus truncates,* Proceedings of the Entomological Society of Ontario vol. 113, pp65-69.

White, N.D.G, Nowicki, T.W., Watters F.L., 1983. Comparison of fenitrothion and malathion for treatment of plywood and galvanized steel surfaces for control of the red flour beetle and the rusty grain beetle, Journal of Economic Entomology, vol.76, n°4, pp856-863.

White, N.D.G, Abramson, D., 1984. Uptake of malathion from galvanized-steel surfaces by stored barley. Journal of Economic Entomology, vol.77, n°2, pp289-293.

White, N.D.G, 1985. Uptake of malathion and pyrimiphos-methyl by rye, wheat, or triticale stored on treated surfaces, Journal of Economic Entomology, vol.78, n°6, 1315-1319.

White, N.D.G, Nowicki, t.W, 1985. Effects of temperature and duration of storage on the degradation of malathion residues in dry rapeseed, J. stored Prod. Res vol 21, n°3, pp111-114.

Study of enzyme-catalyzed biodiesel process with high FFA oil assisted by ultrasonic

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Abstract

The enzyme-catalyzed esterification and transesterification of waste oil with 1-propanol to biodiesel assisted with ultrasonic by an immobilized lipase Novozym 435 was studied. It was found that with the assistant of mild energy and low frequency ultrasonic, the enzyme-catalyzed esterification and transesterification velocities were advanced and higher conversion ratio to propyl oleate was achieved in shorter reaction time compared to conventional mechanical agitation. The optimal reaction conditions were: enzyme amount, 8% of oil weight; molar ratio of propanol to oil 3:1; the temperature of water batch, 40°C~50°C; and the frequency and power of ultrasonic, 28 KHz and 100W. Under such conditions, the conversion ratio achieved was almost 94% in 40 min in comparison with the highest almost 84% conversion ratio achieved. Furthermore, the ultrasonic energy was important to accelerating the speed of diffusing production in reaction system and enhancing the transesterification activity of immobilized lipase.

Key words: biodiesel, high acid value waste oil, lipase, enzyme-catalyzed, 1-propanol, ultrasonic

1 Introduction

Biodiesel consists of fatty acid alkyl esters produced by esterification or transesterification of vegetable oils, animal fats or waste oils with short chain alcohols and is a biodegradable, nontoxic, cleaning and renewable fuel, which is expected as a part of substitute for conventional fossil diesel (Wei du et al., 2004). The use of waste oil as raw material could not only help descending the cost of biodiesel but also have positive significance in the aspect of environmental protection and food security. Moreover, some lipase could be seasoned with the high content of fatty acid in some high acid value waste oil and have particular advantage in enzyme-catalyzed synthesis of biodiesel from high acid value waste oil. However, there also exist two problems that the lipase amount in reaction system was overabundance and the enzyme-catalyzed transesterification time was overlong (Oznur Kose et al., 2002; Yuji Shimada et al., 2002; Hanen Ghamgui et al., 2004; Chen Zhi-feng et al., 2006). However, mild energy and low frequency ultrasonic could not only enhance the diffusing of substrates and produces in the reaction system, but also improve the transesterification activity of immobilized lipase in the water or oil reaction systems.

In the present study, to avoid serious negative effect on lipase by methanol, 1-propanol was selected as acyl acceptor. The enzyme-catalyzed esterification and transesterification of high acid value waste oil with 1-propanol to biodiesel in the conditions of ultrasonic assistant by an immobilized lipase Novozym 435 was studied in this paper. With the assistant of low power 100W ultrasonic, lipase amounts, molar ratio of propanol to oil and the frequency of ultrasonic were investigated gradually. Furthermore, a comparative study was carried out on enzyme-catalyzed esterification and transesterification with the assistant of ultrasonic and with conventional mechanical agitation by itself. At last, the low-temperature property and fluid of the produced propyl oleate biodiesel were mensurated.

2 Materials and methods

2.1 Materials

Candida antarcticaB lipase, immobilized on acrylic resin (Novozym 435), was a gift from Novo Industries (Denmark). The waste oil was from Beijing. All other chemicals were obtained commercially and were of analytical grade.

2.2 Method

2.2.1 Enzyme-catalyzed esterification and transesterificationwith ultrasonic assistant

20g waste oil, certain molar ratio 1-propanol and certain amount immobilized lipase were put into a 100ml three-necked flask fitted with cooler, thermometer and beater. Then, it was heated in a water bath with ultrasonic and continuous whisking and the temperature of water bath was controlled between 40°C~50°C. 0.5ml sample was taken from the reaction mixture at specified times and centrifuged to obtain the upper layer for gas chromatography analysis.

2.2.2 Analytical procedure

2.2.2.1 Account of conversion ratio to propyl oleate

Conversion ratio to propyl oleate in sample = the propyl oleate contents in sample / the propyl oleate contents after completeness esterification

2.2.2.2 Gas chromatography analysis

The propyl oleate contents in sample were quantified using an Agilent 6890N gas chromatography connected to a DB-17HT capillary column (30m × 0.32mm × 0.15um). The column temperature was kept at 160°C for 0.5min, heated to

200°C at 10°C/min, maintained for 1min, heated to 201°C at 0.2°C/min, maintained 0.5min, then heated to 320°C at 50°C/min, then maintained for 5mins. The temperature of the injector and detector were set at 260°C and 330°C, respectively.

3 Results and discussion

3.1 Effect of lipase amount on enzyme-catalyzed transesterification

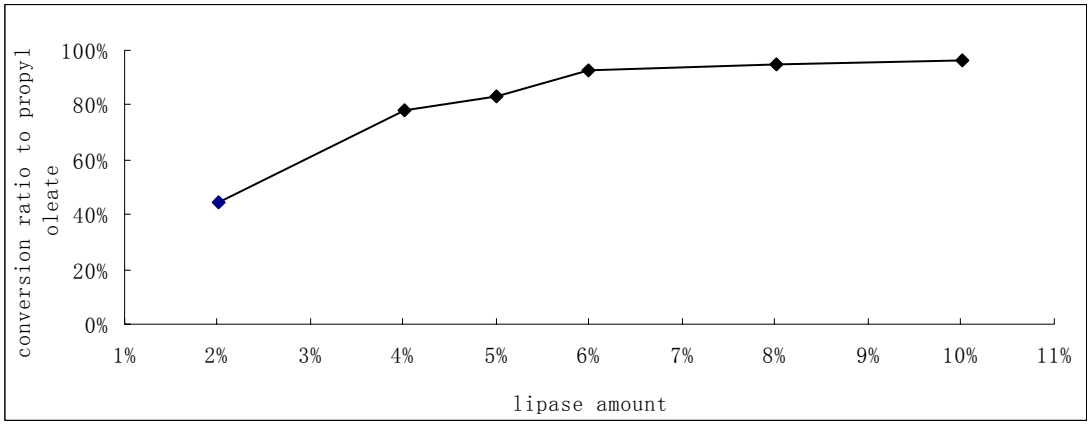

Figure 1 Effect of lipase amount on enzyme-catalyzed transesterification

Label: reaction conditions: 10g waste oil; 0.984g 1-propanol (initiatory molar ratio of propanol to oil 1.5:1), 1.5mol 1-propanol was added twice at 40 min and 1.5h, respectively; lipase amounts: 2%, 4%, 5%, 6%, 8% and 10%; frequency of ultrasonic: 45 KHz; power of ultrasonic: 100W; temperature of water bath: 40°C~50°C; reaction time: 3h.

The amount of immobilized lipase affect directly the velocity of enzyme-catalyzed transesterification and the finally conversion ratio to propyl oleate in the reaction system at some concentration areas. Figure 1 shows the change trend of the finally conversion ratio to propyl oleate in reaction system as lipase amount in reaction system varied. The finally conversion ratio to propyl oleate increased when lipase amounts in the reaction system increased and gradually achieved to equilibrium. When lipase amounts increased from 2% to 6% (based the quality of oil), the finally conversion ratio to propyl oleate increased rapidly from 44.37% to 92.39%. Whereas, when lipase amounts increased from 6% to 10%, the finally conversion ratio to propyl oleate increased slightly. Furthermore, when lipase amounts increased to 8%, the substrates in reaction system were almost saturated by immobilized lipase and the lipase amount did not affect the finally conversion ratio when the lipase amount exceeding 8%. Anyhow, in order to achieve high conversion ratio to propyl oleate and considering saving the lipase, lipase amount was selected as 8% based on oil quantity in the continuative experiments.

3.2 Effect of initiatory molar ratio of propanol to oil on enzyme-catalyzed transesterification

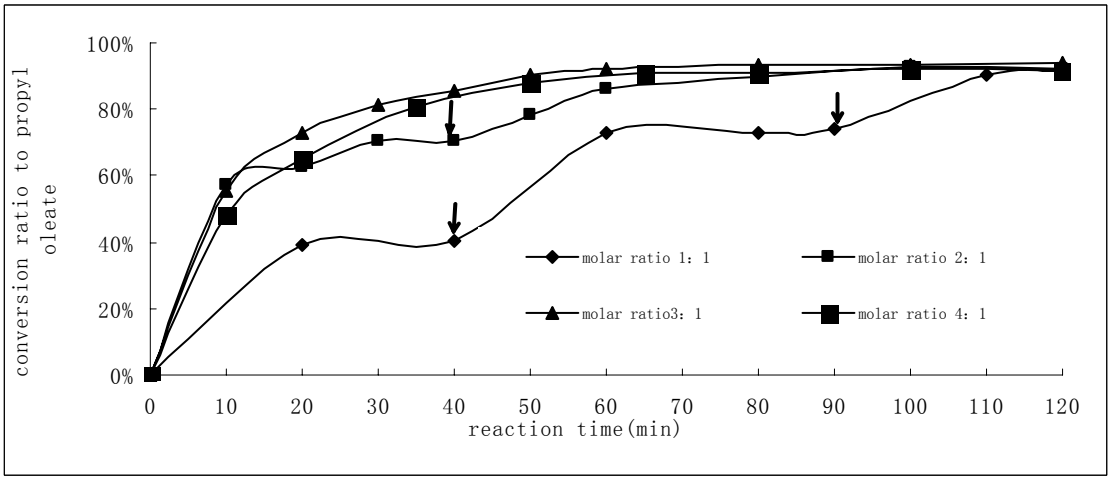

Figure 2 Effect of initiatory molar ratio of propanol to oil on enzyme-catalyzed transesterification

Label: reaction conditions: 20g waste oil; lipase amount: 8% of oil quantity; initiatory molar ratio of propanol to oil: 1:1, 2:1, 3:1, 4:1, and residual 1-propanols were added at the times indicated by arrows; frequency of ultrasonic: 45 KHz; power of ultrasonic: 100W; temperature of water bath: 40°C~50°C; reaction time: 2h.

The variety of initiatory molar ratio of propanol to oil in enzyme-catalyzed reaction system would influence the variety of substrate concentration in the reaction system. In theory, the initiatory molar ratio of propanol to oil was greater, the initiatory substrate concentration was higher, the initiatory velocity of enzyme-catalyzed transesterification was faster, and the finally conversion ratio to propyl oleate would be higher. On the other hand, excessive 1-propanol in the enzyme-catalyzed reaction

system could have negative effect on lipase. Therefore, the exorbitant initiatory molar ratio of propanol to oil was not well. When the initiatory molar ratio of propanol to oil increased from 1:1 to 4:1, the initiatory velocity of enzyme-catalyzed transesterification enhanced obviously; whereas the initiatory velocities of enzyme-catalyzed transesterification at initiatory molar ratio of propanol to oil 2:1 almost was equal to that at initiatory molar ratio of propanol to oil 2:1. Furthermore, when the initiatory molar ratio of propanol to oil increased to 4:1, the velocity of enzyme-catalyzed transesterification descended on the contrary, which was showed in figure 2. Moreover, the conversion ratio to propyl oleate increased in step-shaped fashion when the initiatory molar ratio of propanol to oil was 1:1 and 2:1. It suggested that when the initiatory molar ratio of propanol to oil was 1:1 and 2:1, the content of 1-propanol in reaction system was the limiting factor of enzyme-catalyzed reaction because that the residual 1-propanol was not added into the system timely. On the other hand, when the initiatory molar ratio of propanol to oil increased to 4:1, the content of 1-propanol in the reaction system was excessive and began to cause negative effect on lipase. Therefore, the optimal initiatory molar ratio of propanol to oil was 3:1, which was consistent with the optimal initiatory molar ratio of propanol to oil in theory.

3.3 Effect of the frequency of ultrasonic on enzyme-catalyzed transesterification

The frequency of ultrasonic effecting on enzyme-catalyzed transesterification had some reasons: 1) the frequency of ultrasonic would affect the cavitate effect of ultrasonic. The increase of the frequency of ultrasonic would weaken the cavitate effect of ultrasonic and the existence cycle of cavitated bubble become shorter; then took the edge off the facilitated effect no the diffusion of substrates and produces in the enzyme-catalyzed system consequently. 2) The configurations of immobilized lipase were distinct in different frequencies of ultrasonic because of the change of the bow wave and high speed shoot produced by moment cavitate effect. It was showed in Figure 3 that the initiatory velocity of enzyme-catalyzed transesterification with the frequency of ultrasonic 28 KHz was higher than that with the frequency of ultrasonic 45 KHz in evidence and almost the same finally conversion ratio to propyl oleate with two frequencies of ultrasonic were achieved at last. This phenomenon was explored that the lower frequency of ultrasonic 28 KHz which resulted in stronger cavitate effect was propitious to diffuse substrates and produce in the enzyme-catalyzed system and reduce the concentration of produce in the micro circumstance of immobilized lipase at the primary phases of reaction. At the same time, the lower frequency of ultrasonic 28 KHz may help immobilized lipase form and maintain more reasonable configuration. Therefore, the optimum frequency of ultrasonic was selected as 28 KHz in the continuative experiments.

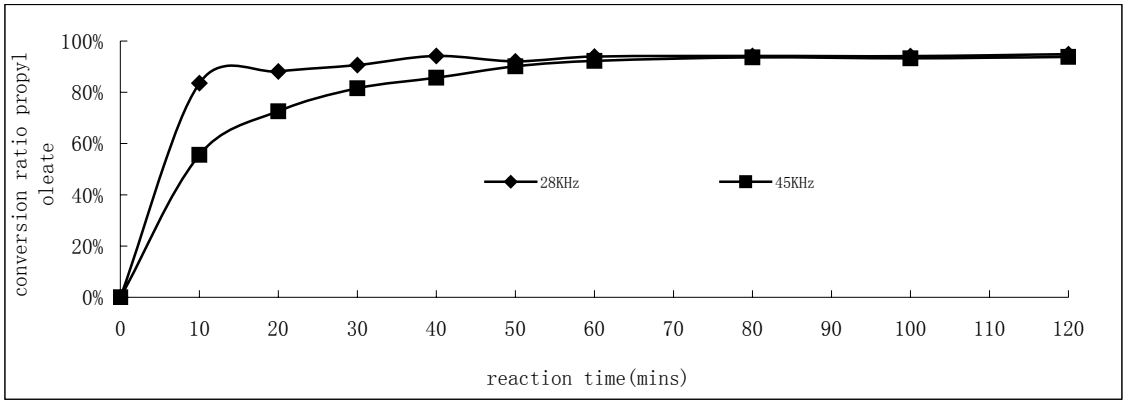

Figure 3 Effect of the frequency of ultrasonic on enzyme-catalyzed transesterification

Label: reaction conditions: 20g waste oil; lipase amounts: 8% of oil quantity; initiatory molar ratio of propanol to oil: 3:1; frequency of ultrasonic: 28 KHz and 45 KHz; power of ultrasonic: 100W; temperature of water bath: 40°C~50°C; reaction time: 2h.

3.4 Intensifying effect of ultrasonic on enzyme-catalyzed transesterification

At the reaction conditions of conventional mechanical agitation, reaction system went equilibrium of conversion ratio to propyl oleate 83%~84% gradually in 60min and the equilibrium of conversion ratio to propyl oleate 83% ~ 84% would not increase any more even if reaction time prolonged to 3.5h, which was showed in Figure 4. It was suggested that at the condition of conventional mechanical agitation the highest conversion ratio to propyl oleate was only 84% because of the saturation effect of produce propyl oleate on immobilized lipase. However, at the condition of ultrasonic, the initiatory velocity of enzyme-catalyzed transesterification enhanced greatly in comparison with that at the conditions of conventional mechanical agitation and the equilibrium of conversion ratio to propyl oleate was achieved rapidly in 40min. It might due to ultrasonic accelerating diffusing substrates and products in the system, avoiding exorbitant concentration of products in the micro circumstances of immobilized lipase, and fit to form immobilized lipase and maintain its reasonable configuration.

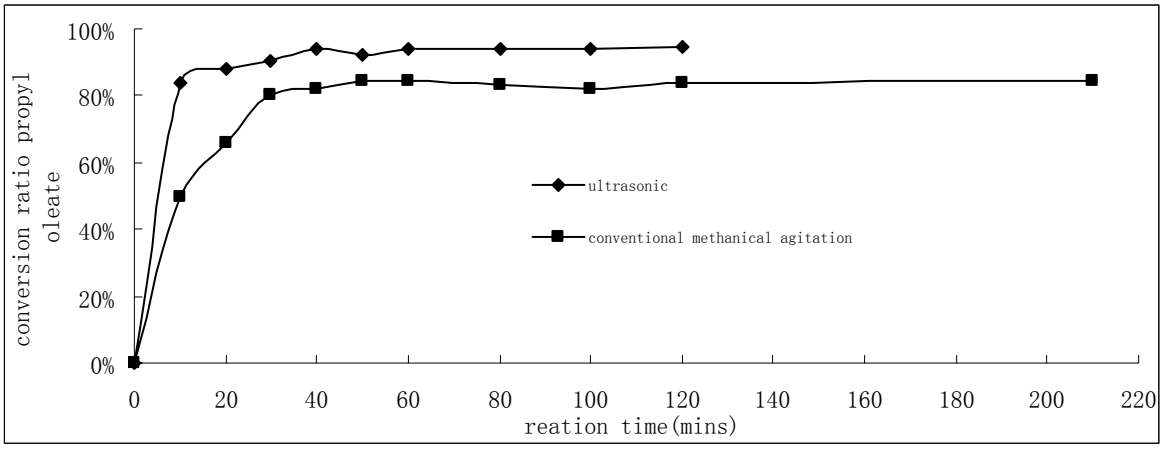

Figure 4 Intensifying effect of ultrasonic on enzyme-catalyzed transesterification

Label: reaction conditions of ultrasonic: 20g waste oil; lipase amounts, 8% of the oil quantity; initiatory molar ratio of propanol to oil, 3:1; the frequency of ultrasonic, 28 KHz; the power of ultrasonic, 100W; temperature of water bath, 40°C~50°C; reaction time, 2h; the reaction conditions of conventional mechanical agitation: 20g waste oil; lipase amounts, 8% based the oil quality; initiatory molar ratio of propanol to oil, 3:1; temperature of water bath, 45°C; reaction time, 3.5h.

4 Conclusions

Immobilized lipase-catalyzed esterification and transesterification of waste oil with 1-propanol is a high efficient biodiesel producing method with the assistant of ultrasonic. In comparison with conventional mechanical agitation, ultrasonic could improve greatly the velocity of enzyme-catalyzed transesterification and the finally conversion ratio to propyl oleate and shorten the reaction time. Accordingly, producing biodiesel carried out expeditiousness producing with the assistant of ultrasonic.

References

Wei du, Yuanyuan Xu, Dehua Liu, et al. Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors [J]. Journal of Molecular Catalysis B: Enzymatic. 2004, 30: 125-129.

Oznur Kose, Melek Tuter, H. Ayse Aksoy, et al. Immobilized Candida antarctica lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium[J]. Bioresource Technology. 2002, 83: 125-129.

Yuji Shimada, Yomi Watanabe, Akio Sugihara, et al. Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing [J]. Journal of Molecular Catatlysis B: Enzymatic. 2002, 17: 133-142.

Hanen Ghamgui, Maha Karra-Chaˆabouni and Youssef Gargouri. 1-Butyl oleate synthesis by immobilized lipase from Rhizopus oryzae: a comparative study between *n*-hexane and solvent-free system [J]. Enzyme and Microbial Technology. 2004, 35: 355-363.

Chen Zhifeng, Wu Hong, Zong Minhua. Transesterification of Waste Oil with High Acid Value to Biodiesel [J]. Chinese Journal of Catalysis. 2006, 27(2): 146~150.

Wang Jian-li, Li Yong-chao, Xu Zhi-chao et al. Effect of ultrasonic on the transesterification of methanol-oil immiscible system [J]. China Oils and Fats. 2006, 31(4): 61-64.

Neuroprotective potential of chronic rapeseed oil diet evaluated by audiogenic seizures test in magnesium-deficient mice.

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Abstract

The magnesium deficiency-dependent audiogenic seizures (MDDAS) test has been validated in adult mice fed for 25-32 days a synthetic 50 ppm Mg-containing diet, rich in omega6 fatty acids, brought by sunflower:corn (1:3) oils. In the present paper, we compared two groups of mice fed either the reference magnesium-deficient diet or a similar diet containing as exclusive lipid source the highly monounsaturated rapeseed oil rich in alphalinolenic acid. This omega 3 rich oil did not change the body weight gain or the magnesium concentration as compared to the omega 6 diet. In contrast, it did not induce the motor hyperactivity observed in the group fed sunflower:corn diet and significantly protected the mice both from audiogenic seizures (50% instead of 100%) and from death since all the convulsive mice survived in the omega 3 group as compared to 50% in the omega 6 group. The MDDAS pattern revealed an increase in the durations of the two first phases (latency and wild running) in the omega 3 group, suggesting that the neuroprotective effect would be mediated mainly through the Na^+ voltage-gated channels. The seizure durations were unchanged whereas the recovery duration tended to decrease suggesting a possible slight antioxidant/anti-inflammatory potential of the rapeseed diet.

Key words: Rapeseed Oil, sunflower:corn oil, omega 6, omega 3, alphalinolenic acids, audiogenic seizure test, magnesium deficit

Introduction

Long-chain polyunsaturated fatty acids (PUFA) are essential components of the central nervous system and are brought in the form of their short chain precursors. In the last decade, many *in vivo* and *in vitro* studies have emphasized the beneficial effect of PUFA, notably omega 3, on cardiac and neuronal excitability. These data suggest that omega 3 supplementation may be of clinical relevance in the prevention of both cardiovascular and brain dysfunctions including epileptic seizures (Lauritzen et al., 2000).

Among omega 3, alphalinolenic acid [18:3n-3 or ALA] which is known to protect against both arythmia and ischemia, represents 9% of the highly monounsaturated (60%) rapeseed oil whereas ALA is absent in omega 6 rich sunflower or corn oils.

The audiogenic seizure test (MDDAS) test has been validated previously (Bac et al., 1998; Maurois et al., 2001) in adult magnesium-deficient mice individually exposed to a calibrated audiogenic stimulus (100dBA, 10kHz, 15 sec). It is characterized by 4 successive phases: wild running latency, wild running, tonic seizure and recovery (or death), the comparative duration of which may be indicative of underlying mechanisms exhibited by the tested compounds. This seizure test is discriminatory, distinguishing between phenytoinergic, GABAergic and ethosuccimide compounds. It is also suitable for evaluation of neuroprotective compounds, namely those presenting antioxidant and/or anti-inflammatory properties (Bac et al., 1998; Maurois et al., 2001).

The aim of the present study was to compare the neuroprotective potential of two magnesium-deficient diets containing either rapeseed or sunflower:corn oils at the same concentration in the MDDAS test.

Materials and Methods

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH, No 85-23, revised 1996).

Animals: Female Swiss OF1 mice (n= 16), 6-weeks old, weighting 20-22g, were purchased from Janvier (Le Genest-St-Isle, France) and were assigned at random to two magnesium-deficient diets (50 ± 5 mg/kg) prepared as previously described (Maurois et al., 1989) containing 5% vegetable oils, either omega 6 (sunflower:corn 1:3) or omega 3 (rapeseed), for 25-32 days. They were placed eight per cage and maintained on a 12:12h light-dark schedule at 21 ± 1°C. They had free access to food and to distilled water which avoids additional magnesium input. In current practice, in order to avoid food oxidation, fresh food was lyophylised and frozen at -20°C. It was given to mice every day in sufficient amount. At the end of the deprivation period, the body weight, serum magnesium concentration, locomotor activity were measured in mice and the MDDAS test was performed.

Plasma magnesium measurement: Magnesium concentrations were determined by atomic spectrophotometry in plasma

and expressed in mg/mL according to Rousselet & Durlach (1971).

Locomotor activity: Mice were transferred individually in an Apelex type 01-1668B actimeter (Bagneux, France). They were allowed to explore for a 2 min period. Their spontaneous activity was measured for another 3 minutes by the crossing of the photocell activity meter and automatically recorded. The experiment was carried out in a sound proof room between 9:00 a.m. and 13:00 p.m. to reduce the confounding influence of diurnal variation in motility.

Magnesium-deficiency dependent audiogenic seizures (MDDAS) test: Individual animals were placed in a 9 dm^3-volume test chamber (30, 20 and 15 cm for length, width and height, respectively) and exposed for 15 sec to an acoustic signal of 10 ± 0.1 kHz frequency and 100 ± 1 dBA intensity. This acoustic stimulus signal was produced by a signal generator and projected via a high frequency speaker mounted on the roof of the chamber. The noise level was measured close to the animal's ear by an external decibel-meter probe. Each animal was subjected to a single audiogenic stimulation. The test measured the capacity of a test compound to provide complete protection against threshold seizures induced by 100 dBA and the audiogenic pattern may indicate the underlying mechanisms exhibited by tested compounds. Audiogenic seizures were videotaped and the duration in seconds of each phase was recorded.

Statistical analysis: Results were expressed as mean ± S.E.M. and analysed by Student's t-test.

Results

At the end of the deprivation period, the plasma level in magnesium-deficient mice was 75% decreased as compared with the initial magnesium levels in both groups (5.70 ± 0.51 and 5.55 ± 0.43 in the omega 6 and omega 3 fed groups respectively *vs* 21.52 ± 1.26 mg/L). The body weight gain was similar in both groups. In contrast, the individual spontaneous locomotor activity, measured for 3 min (Apelex actimeter), showed that magnesium deficiency induced hyperexcitability in the omega 6 group but not in the omega 3 rapeseed group (152.7 ± 37.9 *vs* 97.0 ± 22.5).

In the MDDAS test, the number of convulsive mice was significantly lower in the omega 3 rapeseed group (50%) as compared to the omega 6 sunflower:corn group (100%). In addition, all the mice convulsing in the rapeseed group recovered whereas 50% died in the omega 6 group. The pattern of seizures was also different. The time periods of the first two phases increased significantly ($p<0.05$) in the rapeseed group: latency and wild running durations were 6.7 ± 5.5 and 3.7 ± 0.5 sec instead of 4.0 ± 1.4 and 2.3 ± 0.4 sec respectively in the omega 6 group, while tonic seizures and recovery durations remained similar in the two groups. However the recovery phase showed a non significant tendency to decrease.

Table I : Comparison of the two magnesium-deficient diets on the pattern of MDDAS test

Diets	n	Convulsing mice (%)	Latency (sec)	Wild running (sec)	Tonic seizures (sec)	Recovery (sec)
Rapeseed	8	50	6.7 ± 5.5*	3.7 ± 0.5*	1.7 ± 0.4	46.5 ± 6.7
Sunflower:corn	8	100	4.0 ± 1.4	2.3 ± 0.4	1.8 ± 0.5	43.3 ± 4.1

*significant at $p<0.05$

Discussion

The serum magnesium decrease and the body weight gain were similar in both groups of mice indicating that the two parameters were not dependent on the lipid composition of the diet, in our experimental conditions. In contrast, locomotor activity measurements showed that the omega 6 diet induced hyperexcitability whereas the omega 3 diet did not. Hyperexcitability in magnesium-deficient mice fed the sunflower:corn diet has already been reported (Durlach et al., 2000) whereas the results obtained in magnesium deficient mice fed rapeseed oil have never been described until now. Consequently, it seems that the rapeseed oil exerts a significant neuroprotective effect in mice, which has been also observed in the MDDAS test. Indeed, the rapeseed diet decreased the percentage of convulsive mice and, among them, the number of fatal issues. This result could be ascribed to the high percentage of alphalinolenic acid present in the rapeseed oil, which was shown to prevent neuronal death and brain dysfunctions including epileptic seizures *in vitro* and *in vivo*, in animals treated with kainate or after hippocampal lesions (Vreugdenhil et al., 1996; Xiao & Li, 1999; Lauritzen et al., 2000). In a model of focal brain ischemia in mice, alphalinolenic acid was also shown to confer in addition to neuronal protection, and in agreement with our findings, a long term survival (Heurteaux et al., 2006).

The audiogenic pattern brings additional informations. It appeared clearly that the rapeseed diet increased the first two phase durations as compared to mice fed the sunflower:corn diet. This means that the rapeseed diet increased the seizure threshold in magnesium-deficient mice, as do the phenitoinergic compounds commonly used in the treatment of epilepsy (Bac et al., 1998; Maurois et al., 2001). This effect indicates that the neuroprotective effect of the rapeseed diet would be mainly mediated by Na^+ voltage dependant channels. It can be ascribed to alphalinolenic acid itself but it may be also assumed that the chronic dietary supply of the short-chain essential precursor, alphalinolenic acid, present in the rapeseed oil could lead to a high level of docosohexaenoic acid (DHA), a major compound of the membrane phospholipids of neural tissues (Marszalek JR, et al., 2005). The potent hyperexcitability inhibition observed also by Lauritzen et al. (2000) after injection of ALA has been at least partly ascribed to a decrease in synaptic glutamate transmission, involving the partial inhibition of voltage-sensitive Na^+ and Ca^{2+} channels (Linden & Routtenberg, 1989) and the opening of background K^+ channels which are activated by polyunsaturated fatty acids such as docosahexaenoic and alphalinolenic acid (TREK-1 and TRAAK) (Fink et al., 1998). These channels are abundant in the brain where they are located both pre- and post-synaptically, and are insensitive to saturated fatty acids, which offer no neuroprotection (Lauritzen et al., 2000). K^+ channels activated by polyunsaturated fatty acids might be important in the blockade of glutamatergic transmission by polyunsaturated fatty acids, resulting in a potent

neuroprotective effect in the MDDAS test, since hyperfunction of the glutamatergic system is involved in seizure induction (Bac et al., 1998; Lauritzen et al., 2000).

Finally we paid attention to the slight decrease in the recovery phase duration during with the rapeseed oil diet. This tendency must be confirmed on a greater number of mice, since it may be associated to an anti-inflammatory/antioxidant properties of the rapeseed oil. Indeed, whereas the omega 6 sunflower/corn diet provide linoleic acid which is the precursor of arachidonic acid implicated in pro-inflammatory processes, the omega 3 rapeseed diet provide alphalinolenic acid precursor of DHA fatty acid which are involved in the anti-inflammatory processes (Calder, 2006).

Conclusion

Chronic consumption of rapeseed oil, an alphalinolenic acid rich monounsaturated oil, could help to prevent or reduce neuronal disorders presenting signs or mechanisms observed in magnesium deficient mice, an animal model of audiogenic seizures. The study of this interesting neuroprotective effect of the rapeseed oil, acting as most of the antiepileptic drugs on Na^+ channels, is still in progress and may be of clinical relevance.

References

Bac P., Maurois P., Dupont C., Pagès N., Stables J., Gressens P., Evrard P., Vamecq J. (1998). Magnesium-deficiency-dependent audiogenic seizures (MDDASs) in adult mice: a nutritional model for discriminatory screening of anticonvulsant drugs and original assessment of neuroprotection properties. Neuroscience **18**, 4363-4373.

Calder PC. (2006). n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr, **83**, 1505S-19S.

Durlach J., Bac P., Bara M., Guiet-Bara A. (2000). Physiopathology of symptomatic and latent forms of central nervous hyperexcitability due to magnesium deficiency: a current general scheme. Magnesium Research **13**, 293-302.

Fink M., Lesage F., Duprat F., Heurteaux C., Reyes R., Fosset M., Ladzunski M. (1998). A neuronal two P domain K+ channel stimulated by arachidonic acid and polyunsaturated fatty acids. EMBO Journal **17**, 2397-3308.

Lauritzen I., Blondeau N., Heurteaux C., Widmann C., Romey G., Ladzunski M. (2000). Polyunsaturated fatty acids are potent neuroprotectors. EMBO Journal. **19**, 1784-1793

Linden D.J., Routtenberg A. (1989). Cis-fatty acids, which activate protein kinase C, attenuate Na+ and Ca2+ currents in mouse neuroblastoma cells. Journal of Physiology **419**, 95-119.

Heurteaux C., Laigle C., Blondeau N., Jarretou G., Ladzunski M. (2006). Alpha linolenic acid and riluzole treatment confer cerebral protection and improve survival after focal brain ischemia. Neuroscience **137**, 241-251.

Marszalek JR., Lodish HF. (2005). Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: breastmilk and fish are good for you. Annu Rev Cell Dev Biol. 21, 633-57.

Maurois P., Gueux E., Rayssiguier Y. (1989). Protective effect of severe magnesium deficiency on *Plasmodium chabaudi* infection. Magnesium Research **2**, 183-187.

Maurois P., Bailly F., Pagès N., Bac P., Fourniat J., Dupont C., Bernier J.L, Catteau J.P., Stables J.P., Vamecq J. (2001). The multiple facets of the MDDAS test: application to the detection of neurotoxic molecules or neuroprotective (anti-inflammatory and/or antioxidant) molecules in central nervous hyperexcitability induced by severe magnesium deficiency. In: Nutrition and Health, J. Rayssiguier, A. Mazur, J. Durlach (eds). John Libbey and Co, Londres, 434-437.

Rousselet F., Durlach J. (1971). Techniques analytiques et explorations pratiques du métabolisme magnésique en clinique humaine. In Duralch J, editor, 1er Symposium International sur le Déficit Magnésique en Pathologie Humaine. I. Vol. des rapports, SGEMV ed., Vittel 65-90.

Vreugdenhil M., Bruehl C., Voskuyl R.A., Kang J.X., Leaf A., Wadman W.J. (1996). Polyunsaturated acids modulate sodium and calcium currents in CA1 neurons. Proceedings of the National Academy of Sciences USA **93**, 12559-12563.

Xiao Y.F., Li X. (1999). Polyunsaturated fatty acids modify mouse hippocampal neuronal hyperexcitability during excitotoxic or convulsant stimulation. Brain Research **846**, 112-121.

High-laurate canola oil in production of structured lipids

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Abstract

Structured lipids (SL) containing lauric acid and long-chain polyunsaturated fatty acids (LC PUFA) were produced using high-laurate canola oil (Laurical 35) and eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) as substrtaes. Laurical 35, containing 37% lauric acid and 34% oleic acid, was subjected to acidolysis reaction in the presence of enzyme catalysts from *Mucor miehei, Pseudomonas sp*., and *Candida rugosa*. The process variables for optimization of incorporation of LC PUFA into Laurical 35 were the amount of enzyme (2-6%), reaction temperature (35-55°C) and incubation time (12-36 h). The maximum incorporation of EPA, DPA and DHA into Laurical 35 were 62.2, 50.8 and 34.1% under conditions (enzyme concentration 4.36-5.41%, temperature of 38.7-43.7°C and reaction time of 23.9-44.7 h. Both LC PUFA and lauric acid were mainly esterified to the sn-1,3 positions of the modified oils. The modified oil was found to be less stable than the original oils because of its higher degree of unsaturation and removal of active antioxidants from the original oils. The resultant products containing both medium-chain fatty acid (C12:0) and LC PUFA may serve as nutraceutical and functional food ingredients.

Key words: Acidolysis, EPA, DPA, DHA, high-laurate canola oil, lipase, medium-chain fatty acids, ω3 fatty acids, positional distribution, response surface methodology, structured lipids, oxidative stability, conjugated dienes (CD), thiobarbituric acid reactive substances (TBARS).

Introduction

Specialty lipids include a wide range of products amongst which structured lipids are a main class. Structured lipids (SL) are triacylglycerols (TAG) or phospholipids (PL) in which fatty acids are placed in specific locations in the glycerol backbone and are produced using a chemical or enzymatic process. Much attention has been paid to SL due to their potential biological functions and nutritional perspectives. Designing SL with selected fatty acids at specific locations of the TAG for medicinal application has attracted much attention. The position of fatty acids (FA) in the TAG molecules (*sn*-1, *sn*-2, and *sn*-3) would have a significant impact on their metabolism in the body. In general, FA at the terminal positions of TAG (*sn*-1 and *sn*-3) are hydrolyzed by pancreatic lipase and absorbed while those at the middle position of TAG (*sn*-2) remain unchanged and are used in the synthesis of new TAG. For example, it may be desirable to develop a SL containing LC PUFA at the *sn*-2 position with medium-chain fatty acids (MCFA) at the *sn*-1,3 positions for patients with mal-digestion as well as cystic fibrosis.

Recognition of the health benefits associated with consumption of seafoods (n-3 fatty acids) is one of the most promising developments in human nutrition and disease prevention research in the past three decades. Long-chain n-3 fatty acids present in seafoods and algal sources include eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3). DPA has not been studied in much detail because of availability problems as it is present in a much lower concentration in marine oils as compared with EPA and DHA. It is also difficult to purify it from mixtures containing EPA and DHA which have similar physico-chemical properties (Yazawa, 2001).

High-laurate canola oil was produced by Calgene's Inc. (Davis, CA) in order to provide an alternate to several palm kernel oil fractions (Del Vecchio, 1996). In this study, the ability of different lipases to catalyze the acidolysis of high-laurate canola oil (Laurical 35) with EPA, DPA, and DHA was explored. Effects of enzyme amount, reaction temperature and time on the incorporation of LC PUFA into Laurical 35 using response surface methodology (RSM), were also investigated. The oxidative stability of the resultant structured lipids (SL) was then assessed.

Material and Methods

Methods

Acidolysis Reaction. The reaction mixture contained Laurical 35 (70 mg), fatty acid to Laurical 35 at a mole ratio 3:1, enzyme amount (2-6%), water (2%), and hexane (3.0 mL). The mixture was kept at temperatures ranging from 35 to 55°C for 12 to 36 h.

Preparation of Fatty Acid Methyl Esters (FAMEs). Fatty acid profiles of products were determined following conversion to their corresponding methyl esters (FAMEs) and these were analysed by gas chromatography.

Stereospecific analysis. Positional distribution (sn-2 and sn-1+ sn-3) of fatty acids in modified and unmodified Laurical 35 was carried out with standard methods using gas chromatography and enzymatic reactions.

Experimental Design for Response Surface Analysis. Experimental design for response surface analysis was performed as

described in our previous studies (Hamam and Shahidi, 2006 [a,b]).

Oxidative stability tests. The stability of the products was tested using standards measures of oxidation (conjugated dienes, CD; and thiobarbituric acid reactive substances, TBARS).

Results

The fatty acid profile of high-laurate canola oil (Laurical 35). The original oil contained 37.0% lauric acid as well as oleic (33.9 %) and linoleic (3.35 %) acids.

Enzyme screening. Lipases from *Candida rugosa, Mucor miehei,* and *Pseudomonas sp.* were most effective, for incorporating EPA, DPA, and DHA into Laurical 35, respectively.

Positional distribution. Positional distribution analysis of modified Laurical 35 revealed that both lauric acid and LC PUFA (EPA and DHA) were located at the sn-1 + sn-3 positions of the modified oils. Meanwhile DPA was randomly distributed over the three positions of the modified Laurical 35 with DPA.

Response Surface Methodology. Table 1 shows critical values for the three factors (enzyme load, reaction time and temperature) examined. The maximum incorporation of EPA (62.2%) into Laurical 35 was predicted at 4.36% enzyme load at 43.2°C over 23.9h. The stationary point for the degree of DPA incorporation (%) into Laurical 35 reached a maximum of 50.8% at 5.41% enzyme concentration, and 38.7°C in 33.5 h. Similarly, the maximum incorporation of DHA (34.1%) into Laurical 35 was obtained when enzyme amounts, reaction temperature and time were 5.25%, 43.7°C, and 44.7h, respectively. EPA proved to be more reactive than DPA or DHA. EPA has five double bonds while DHA has six. The more double bonds the chain has in the cis configuration, the more bent it is. Since DHA has six cis double bonds, it becomes quite curved compared to EPA and hence DHA has more steric hindrance than EPA. DPA and DHA have the same chain length and both belong to the n-3 family. However, DPA has one less double bond than DHA and hence less steric hindrance Therefore, the structural differences (chain length, number and location double bonds) between these three molecules had a marked effect on their incorporation into Laurical 35.

Oxidative stability tests. The present results indicate that modification of Laurical 35 LC PUFA resulted in its lower stability as evidenced by both CD and TBARS, due to the incorporation of highly susceptible FA, DHA or DPA or EPA, to oxidation. Another possible explanation could be attributed to the formation of tocopheryl esters from reaction of free carboxylic acids in the medium and tocopherols present in the oil during the process of preparation of SL and these do not render any stability to the resultant modified oils (Hamam and Shahidi, 2006).

Table1. Canonical analysis of response surface for acidolysis of high-laurate canola oil with EPA or DPA or DHA

Factor	Laurical 35, EPA (%)	Laurical 35, DPA (%)	Laurical 35, DHA (%)
Amount of enzyme (w %, X1)	4.36	5.41	5.25
Reaction temperature (C, X2)	43.2	38.7	43.7
Reaction time (h, X3)	23.9	33.5	44.7
Stationary point	maximum	saddle	saddle
Predicted value [a]	62.2	50.8	34.2
Observed value [b]	61.1 ± 0.95	48.7 ± 0.62	38.8 ± 3.11

[a] Predicted using the polynomial model

[b] Mean value of triplicate determinations ± standard deviation

Conclusions

Lipases from *Candida rugosa, Mucor miehei,* and *Pseudomonas sp* were most effective, for incorporating EPA, DPA, and DHA into Laurical 35, respectively. RSM showed that the maximum incorporation of EPA (62.2%) into Laurical 35 was possible using 4.36% enzyme, at 43.2°C and over 23.9h. In Laurical 35-based SL, maximum incorporation of DHA (34.3%) was achieved at a 5.25% enzyme, at 43.7°C, over 44.7 h. The maximum incorporation of DPA into Laurical 35 (50.8%) was obtained when enzyme amount, reaction temperature and time were 5.41%, 38.7°C and 33.5 h, respectively. Incorporation of LC PUFA into Laurical 35 was in the order EPA>DPA>DHA. EPA or DHA as well as lauric acid were mainly esterified to the sn-1,3 positions of the modified oils. Enzymatically modified Laurical 35 with EPA, DPA, and DHA had a higher conjugated dienes (CD) value than the unmodified starting material. Modified Laurical 35 with DPA was less stable than DHA- or EPA- modified Laurical 35 as reflected in both CD and TBARS values.

References

Del Vecchio, A.J. (1996). High-laurate canola oil: How Calgene's program began, where it's headed. INFORM **7,** 230-240.

Hamam, F., Shahidi F. (2006a). Synthesis of structured lipids containing medium-chain and omega-3 fatty acids. J. Agric. Food Chem. **54,** 4390-4396.

Hamam, F., Shahidi F. (2006b). Acidolysis reactions lead to esterification of endogenous tocopherols and compromised oxidative stability of modified oils. J. Agric. Food Chem. **54**, 7319-7323.

Yazawa, K. (2001). Recent development of health foods enriched with DHA, EPA and DPA in Japan. World Rev. Nutr. Diet. **88**, 249-252.

Study on preparation of conjugated linoleic acid

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Abstract

Conjugated linoleic acid has good physiological functions. The reactive mechanism of conjugation catalyzed by alkali and the factors affecting the conjugation such as reaction time and alkali dosage were discussed. The optimal conditions were obtained by orthogenesis test, which resulted in a conjugation yields above 90%.

Key words: linoleic acid; alkali catalizatioin; conjugation

Introduction

Conjugated Linoleic Acids (CLA) were first reported in 1987 (Ha et al., 1987). It is a term used to describe a group of positional and geometric isomers of linoleic acid in which the two double bonds are conjugated (Ha, et al.,1989). It has many kinds of physiological activities such as anticarcinogenic and anti-atheromatous, participating on the disassemble and metabolism of fat, increasing body immunity (Zhang et al., 2000).

Natural CLA is found in meat and milk of ruminant such as cattle and sheep. It is seldom in other animal and vegetable oils. So artificial synthesis is of importance. There were many methods for the synthesis, such as dehydration of allylic hydroxy oleate, alkali catalyzation and enzyme catalyzation (Chen et al., 2000). Among these methods, alkali catalyzation is the most efficient one. In this study, the reactive mechanism and optimal conditions of alkali catalyzing method were studied.

Material and Methods

Materials: Sunflower oil was purchased from Inner Mongolia. NaOH and H_2SO_4 were obtained from Luoyang Chemical Co. Inc. Glycol and hexane were purchased from Tianjin Chemical Co. Inc.

Alkali-catalyzed conjugation (Nie et al.,1997) : Sunflower oil, hexane and alkali were transferred to three joint flasks with a condenser, an agitator, a temperature sensor and air intaker. Temperature sensor should be protected from the solution by vitreous duct with bottom closed. The reaction was performed under nitrogen. The product was cooled to about 40°C, neutralized by oil of vitriol and water fluid. The topper was separated and dried by sodium sulfate and conserved in refrigerator.

Fatty acid analysis: Fatty acids of alkali-catalyzed product were converted to the corresponding fatty acid methyl esters with a mixture of 14% BF_3 in methanol and toluene (1:1,v/v) under nitrogen at 90°C for 45min. Fatty acid methyl esters were analyzed on a flexible silica capillary column with an Angelent 5980N gas chromatograph, equipped with a flame-ionization detector and an automated injector. Column temperature was programmed from 180 to 220°C at a rate of 1°C/min and then held for 10 min. Injector and detector temperatures were set at 250 and 300°C, respectively. Hydrogen was used as the carrier gas at a head pressure of 15 psi.

Results

Composition of sunflower oil

Table 1 The fatty acid composition of sunflower oil

Fatty acid	Palmic acid	Stearic acid	Oleic acid	Linoleic acid	Linoleinic acid
Content	6.87	5.38	22.94	63.73	0.56

Linoleic acid is the main fatty acid in sunflower oil (Table 1). Five carbons of the double bonds in linoleic acid are in a plane, so the trans-construct has low energy. The product of alkali-catalyed conjugation is mostly the formation of cis-trans. 9c,11t-18:2 and 10t,12c-18:2 acids account for above 70%. And 9c,11t-18:2 has be proved to have physiological activities.

There were many other reactions along with conjugation. So it is difficult to reflect the rule of the reaction. In this test, palmic acid that is steadiness in the reaction was used as contrast. Relative concentration of other fatty acid is the ratio between the content of a particular acid and the content of palmic acid.

Effect of time on the reaction

The test was performed at 190°C with a solvent dosage of 3 times of sunflower oil volume and alkali dosage of 1.4 times of the saponification equivalent of sunflower oil. As shown in Fig 1, with the time prolonged, oleic acid content did not change. The content of CLA increased rapidly within 3 hours and reached the peak at 5 hour. After 5 hour, little change could be

detected. When the reaction time was extended, the amount of byproduct increased too, thus the physiological activities should be decreased. In sunflower oil, the content of linoleinic acid is below 1%. So the influence of linoleinic acid on the reaction is neglectable. Hence, 4-5 hours is suitable for the reaction.

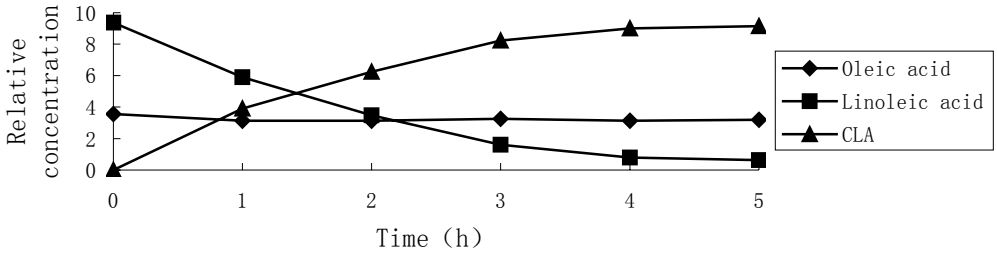

Fig 1 The effect of time on reaction

Effect of alkali amount on the reaction

With the increase of alkali amount, the content of oleic acid did not change (Fig 2). When the alkali dosage was 1.1 times of saponification equalivent, the conversion rate of linoleic acid was very low. When it ws 1.7 times, the conversion rate reached the peak. Continuous increase of the alkali dosage did not result in any change of the conversion rate. The reaction turned slower if the alkali dosage was only equal to saponification equalivent, because saponification is easier than conjugation. As a catalyzer, when alkali concentration reached a certain value, the effective collision among molecules reached the peak. After that, increase of the catalyzer will not change the effective collision.

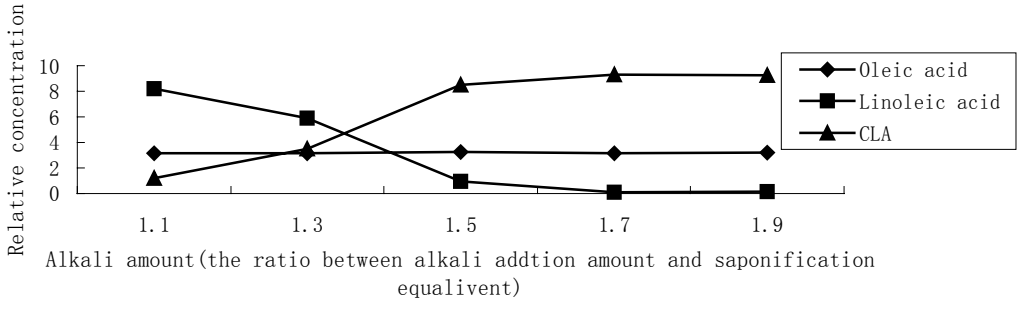

Fig 2 The effect of alkali amount on the reaction

Effect of solvent dosage on the reaction

It was observed that superfluous foam occurred when solvent dosage is very small, for example, at 1:2 (v/v). It would be difficult to separate after conjugation and cause the lose of CLA significantly. If the solvent dosage is very high, it will decrease the effective collision,results in the decrease of the conjugation.

Selection of optimal condition

According to the single factor experiment, four factors and three levels was designed as Table 2, 3,4.

Table 2 Factors and levels of orthogonal experiment

	A:Temperature (°C)	B:Time (h)	C:Ratio between alkali amount and saponification equalivent	D:Ratio between volumes of sunflower oil and solvent
1	190°C	1	1.7	1:7
2	170°C	7	1.5	1:3
3	boiling	4	1.3	1:5

Alkali amount and time has observably effect on the reaction. The optimal condition was A (boiling),

B (7h), C (1.7), D (1:5) in Table 3. It can be calculated that the optimal condition is A (190°C), B (4h), C (1.7), D (1:3). Its conjugation rate was 99.5% under the condition.

Conclusions

Alkali-catalyed conjugation of oil is a complex reaction. Under proper conditions, conjugation is taking place mainly. The content of linoleic acid in sunflower oil is high. The optimal condition of alkali-catalyed conjugation is that alkali dosage of 1.7 times of saponification equalivent, with solvent volume 3 times of sunflower oil, and the reaction for 4 hours at 190°C.

Table 3 The condition of orthogonal experiment

	A	B	C	D	Relative concentration		Average value	Convertion rate
1	1	1	1	1	6.14	5.68	5.91	63.8%
2	1	2	2	2	9.21	9.15	9.18	99.1%
3	1	3	3	3	6.06	6.38	6.22	67.1%
4	2	1	2	3	4.85	4.77	4.81	51.9%
5	2	2	3	1	2.44	2.1	2.27	24.5%
6	2	3	1	2	8.46	8.32	8.39	90.6%
7	3	1	3	2	3.64	3.42	3.53	38.1%
8	3	2	1	3	9.3	9.22	9.26	99.9%
9	3	3	2	1	8.49	8.41	8.45	91.2%
I	42.62	28.5	47.12	33.26				
II	30.94	41.42	44.88	42.2				
III	42.48	46.12	24.04	40.58				
R	11.68	17.62	23.08	8.94				

Table 4 Analysis of variance on date of orthogonal experiment

Soruces of variation	A	B	C	D	S_{e1}^2	S_{e2}^2	S_e^2
Sum of square	14.9785	27.7489	54.0005	7.5628	22.5413	0.1634	22.7047
df.	2	2	2	2	4	9	13
Mean squared deviation	7.4893	13.8745	27.0003	3.7814	5.6762	/	/
F value	4.2881	7.9441	15.4595	2.1651	$F_{0.95}$ (2, 13) =3.80		
Significance	*	* *	* *	/	$F_{0.99}$ (2, 13) =6.70		

References

Chen Zhongzhou,LI Yanmei,Zhao Gang,Zhao Yufen(2000). China Oils and Fats 25:41-45

Ha Y L, Grimm N K, Pariza M W(1987). Anticarcinogens from Ground Beef Heat-Altered Derivatives of Linoleic Acid. Carcinogenesis 8:1881-1887

Ha Y L, Grimm N K, Pariza M W(1989). Newly Recognised Anticarcinogenic Fatty Acid: Identilication in Natural and Processed Cheeses. J. Agric. Food Chem 37:75-81

Nie Xiaoan,Wang Ding xuan(1997). Research on the Isomerization Principle of Unsaturated Fatty Acids or Their Methyl Esters (II)—the Base Isomerization of Oils 22:46-48.

Zhang Genwang, Yang Tiankui, Guo Zheng(2000). Study on Biological Activity Substance Conjugated Linoleic Acid(CLA)-Physiological Activity and Application Prospect of CLA.China Oils and Fats 25: 13-16

The effects of extrusion on tannin content in rapeseed meal

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Abstract

In the paper, tannin content was employed as evaluation index; heating temperature of extruder sleeve, moisture of extruder feed material and rotation speed of extruder screw were considered. Three factors and three levels were implemented into the combined test design of quadratic orthogonal rotation, a regression equation and single factor graphical analysis were used to demonstrate the rule of influential factors on test indices, and investigate the contribution rate of test factors to index factors. The above built a theoretical basis for the comprehensive utilization of extrusion technology in the processing by-products of rape.

Keywords: tannin, test design, extrusion

Introduction

Rapeseed meal contains 28%~ 45% proteins, its biological value PER of amino acid is superior to that of soybean protein and is close to the value recommended by WHO/FAO, and it is a full protein. Therefore, research on the protein extracted from rapeseed meal (Xu, L., and Dio-sady., 1994), the confirmation of proteolytic conditions (Pang Guangchang, 1999) as well as acylated polypeptide & compound amino acid made from rapeseed protein (Jacques G., 1990; Wang Zhijun, 2001), etc. has been carried out in foreign countries with obvious progress. The anti-nutritional factors such as glucosinolate, tannin and phytic acid in rapeseed meal limit the extraction and utilization of this excellent protein source. The adverse effects of toxic glucosinolate on rapeseed protein have been well removed by breeding, however, the content of anti-nutritional factors of tannin and breeding now cannot reduce phytic acid, etc. So, a substrate processing method by which the protein recovery rate can be enhanced and the content of anti-nutritional factors of tannin and phytic acid, etc. can be reduced at the same time is to be found. Extrusion puffing is a high technology. Used in the extrusion of rapeseed meal, it can deactivate the anti-nutritional factors of tannin and phytic acid, etc., release the protein combined with tannin and phytic acid, and cause protein denaturation. All of these help the hydrolase action and enhance the protein recovery rate. In this paper the variation rule of tannin content in rapeseed meal under different extrusion conditions was mainly discussed. The extrusion processing of substrate to enhance proteolytic efficiency and deactivate phytic acid will be reported in another paper.

Material and Methods

Materials and Reagents

Rapeseed meal (provided by Hubei Huayi Oil Technology Co., Ltd.; moisture 10.34%, protein 42.48%, tannin 1.21% and phytic acid 3.66%), isatin, $KMnO_4$, bone black powder and concentrated H_2SO_4 etc. All the reagents belong to AR level.

Apparatus

DS32-II Single Screw Extruder (Shandong Saixin Inflating Machinery Co., Ltd.), DE110 Electronic Analytical Balance (Changshu Weighing Apparatus Factory of China Light Industry Machinery General Corporation), HH·SY11-Ni 4B Thermostat Water Bath (Beijing Changfeng Instrument and Meter Co., Ltd.), HG101-1 Electric Blast Drying Oven (Nanjing Laboratory Apparatus Factory), DWF-100 Electric Plant Crusher (Scientific Research Instrument Factory of Huangye City, Hebei Province), LNK-872 Versatile Fast Digester (Research Institute of Scientific and Educational Instrument, Yixing City, Jiangsu Province), LNK-871 Kjeldahl Nitrogen Determination Apparatus (Research Institute of Scientific and Educational Instrument, Yixing City, Jiangsu Province), 78-1 Magnetic Stirrer (Jiangsu Jintan Medical Instrument Factory), etc.

Methods

Determination of Tannin Content in Rapeseed Meal

Potassium permanganate titration is used. Tannin can be absorbed active bone coal and oxidized by potassium permanganate. Tannin content can be calculated according to the difference between oxidizing values before and after absorption reaction. Isatin is oxidized by potassium permanganate, turning from blue to yellow so as to indicate the end.

Respectively suck up 0, 1, 3, 5, 10 and 15mL from 400μg/mL tannin standard solution (equivalent to 0, 400, 1200, 2000,

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4000 and 6000μg of tannin content), determine them by the newly prepared $KMnO_4$ solution, and draw the following standard curve as Figure 1.

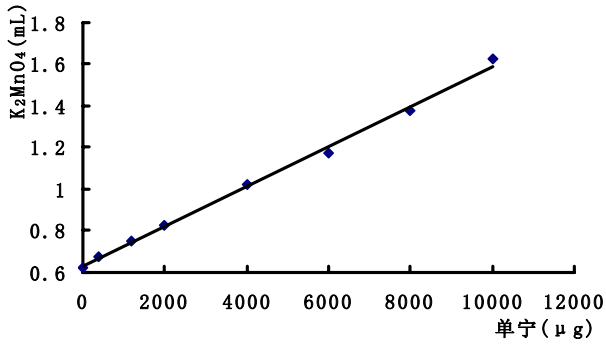


Figure 1. The Standard Curve of Tannin Determination

Design

Table 1. Code Table of Factors and Levels

Level	X_1 Temperature (°C)	X_2 Screw Speed ($r.min^{-1}$)	X_3 Moisture (%)
-1.682	33	166	17
-1	40	180	20
0	50	200	25
1	60	220	30
1.682	67	234	33

The regression equation between K_2MnO_4 and tannin content is obtained by the standard curve:

$$Y=1 \times 10^{-4}X+0.6288;\ R2=0.9968$$

Calculate the tannin content of rapeseed meal under different extrusion conditions by the regression equation.

Table 2. Implementation Plan and Test Date

No.	X_1	X_2	X_3	Y tannin (%)
1	1	1	1	0.9056
2	1	1	-1	0.7360
3	1	-1	1	0.8671
4	1	-1	-1	0.7895
5	-1	1	1	0.8616
6	-1	1	-1	0.7915
7	-1	-1	1	0.8934
8	-1	-1	-1	0.8737
9	1.682	0	0	0.7783
10	-1.682	0	0	0.8031
11	0	1.682	0	0.6313
12	0	-1.682	0	0.5719
13	0	0	1.682	0.8744
14	0	0	-1.682	0.8896
15	0	0	0	0.8071
16	0	0	0	0.7391
17	0	0	0	0.6847
18	0	0	0	0.7973
19	0	0	0	0.8066
20	0	0	0	0.7035
21	0	0	0	0.7973
22	0	0	0	0.8066
23	0	0	0	0.8723

Note: X_1-temperature of extruded material, X_2-rotation speed of extruder screw, X_3-moisture of feed material

Based on the relevant documents and reports (Zhu Zeng, 1995; Shen Dechao, 1999) and combined with the actual conditions of existing extruders, select three factors, i.e. temperature of extruder sleeve, moisture of feed material and rotation

speed of extruder screw, as extrusion parameters, at the same time, select five levels, and use the quadratic orthogonal rotation combination to design and arrange the test (Xu Zhongru, 1988). See Table 1 for Factor Level coding Table. Perform data analysis and graphic processing and research on the rule of effect of each parameter on tannin content in investigation index-rapeseed dregs with computer software.

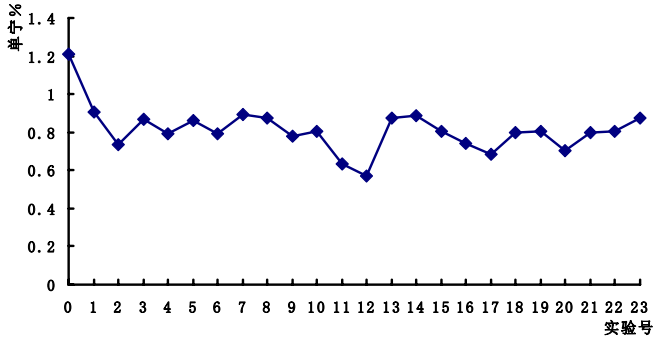


Figure 2. Changes of Tannin Content under Different Test Conditions

Results and Discussion

Changes of Tannin Content under Different Test Conditions

Figure 2 indicates the tannin content in rapeseed meal as raw material and the tannin content corresponding to each test number in Table 2.

The Regression Equation with Tannin Content as Evaluation Index

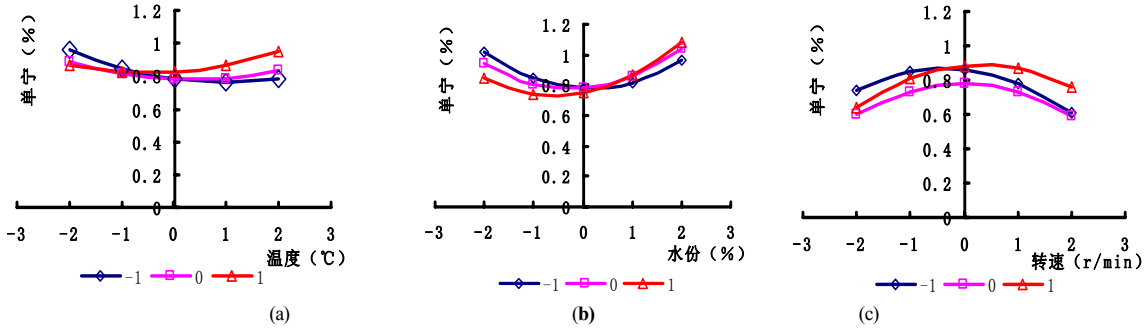
The test data are processed by computer to get the regression equation with tannin content as evaluation index:

$$Y=0.778-0.012X_1-0.002X_2+0.023X_3+0.021X_1^2+0.012X_1X_2+0.020X_1X_3-0.046X_2^2+0.018X_2X_3+0.053X_3^2$$

The following is the variance analysis table of regression equation

Table 3. Variance Analysis Table

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	Critical Value
Regression	D_r=0.102	f_r=9	0.0113	F_2=4.9982	$F_{0.1}(9,13)$=2.16
Surplus	D_s=0.061	f_s=13	0.0047		
Fitting	D_f=0.033	f_f=5	0.0066	F_1=3.6159	$F_{0.05}(5,8)$=3.69
Error	D_e=0.028	f_e=8	0.0035		
Total	D_t=0.163	f_t=22			



(a) (b) (c)

Figure 3. Single Factor Analysis of Tannin Content

The Factor Contribution Rate of the effects of Extruder Parameters on the Tannin Content in Rapeseed Meal.

The model shows that the contribution rates of the effects of factors on tannin content are respectively: material moisture $X_3$1.888> rotation speed of extruder screw $X_2$1.310 > temperature of extruder sleeve $X_1$1.379.

Discussion

The Extrusion Effects on Tannin Content in Rapeseed Meal

Table 2 and Figure 2 indicate that the tannin content in rapeseed meal varies with the extrusion conditions, the tannin content in 23 groups of material under different extrusion conditions is all lower than that in the raw material, and the tannin content of No.12 sample is even reduced to nearly 50% of that in the raw material. So extrusion obviously deactivates tannin.

Variance Analysis of Regression Equation

In Table 1, $F_1<F_{0.05}$ (5,8) =3.69, which shows that the regression equation is well fit; $F_2>F_{0.1}$ (9,13) =2.16, which shows

that the equation is obvious in 0.1 level, i.e. the test data substantially matches the mathematical model.

Single Factor Analysis of the Effects of Test Factors on Indices.

Figure 3 is the dimension reduction analytic map for the affects of extrusion parameters on the investigated indices under -1,0,+1 level conditions.

Figure 3 (a) is the curve in which the tannin content in rapeseed meal varies with X_1 when X_2 and X_3 are respectively fixed at -1, 0, +1 level. The dimension reduction analysis equations corresponding to different level values are respectively $f(x_1) = 0.0212x_1^2-0.0440x_1+0.7826$, $f(x_1) = 0.0212x_1^2-0.0120x_1+0.7776$, $f(x_1) = 0.0212x_1^2+0.0201x_1+0.8239$. Figure (a) indicates that the tannin content changes with the temperature of extruder sleeve substantially in the same way in case of other factors fixed at different levels. As the temperature of extruder sleeve rises, the tannin content first falls and then rises, without great fluctuation. The graphical analysis indicates that the temperature of extruder sleeve has little impact on the tannin content, and is a minor parameter influencing the changes of tannin content.

Figure 3 (b) is the curve in which the tannin content in rapeseed meal varies with X_2 when X_1 and X_3 are respectively fixed at -1, 0, +1 level. The dimension reduction analysis equations corresponding to different level values are respectively $f(x_2) = -0.0456x_2^2-0.0323x_2+0.8612$, $f(x_2) = -0.0456x_2^2-0.0021x_2+0.7776$, $f(x_2) = -0.0456x_2^2+0.0280x_2+0.8828$. Figure (b) indicates that the tannin content changes with the rotation speed of extruder screw substantially in the same way in case of other factors fixed at different levels. As the rotation speed of extruder screw rises, the tannin content first rises and then falls, with obvious changing trend. It is because: as the rotation speed rises, the residence time of material in the chamber is shortened, tannin is less deactivated, and its content rises; as the rotation speed go on rising, the shearing action of screw on material is strengthened, the strengthened degree of tannin due to deactivation is higher than the weakened degree due to the reduced residence time of material, so the tannin content falls.

Figure 3 (c) is the curve in which the tannin content in rapeseed meal varies with X_3 when X_1 and X_2 are respectively fixed at -1, 0, +1 level. The dimension reduction analysis equations corresponding to different level values are respectively $f(x_3) = 0.0535x_3^2-0.0147x_3+0.7797$, $f(x_3) = 0.0535x_3^2+0.0228x_3+0.7776$, $f(x_3) = 0.0535x_3^2+0.0603x_3+0.7514$. Figure (c) indicates that the tannin content changes with the moisture of extruded material in the same way in case of other factors fixed at different levels. As the moisture of material rises, the tannin content first falls and then rises. It is because: proper moisture is helpful to the instantaneous puffing of material being extruded from the die orifice, and tannin is more deactivated; as the material moisture go on rising, the fluidity of extruded material gets better, the residence time in extruder chamber is shortened, and tannin is less deactivated.

Analysis of Factor Contribution Rate

The values of contribution rates indicate: The two factors of the moisture of extruded material and the rotation speed of extruder screw have greater influence on the changes of tannin content in rapeseed meal; the temperature of extruder sleeve has less influence on the changes of tannin content, which can be regarded as a minor factor in production. The analysis of factor contribution rate basically corresponds with the conclusion of graphical analysis.

Conclusions

The results indicate that extrusion obviously deactivates tannin, a resistance factor in rapeseed meal affecting protein absorption. The tannin content of rapeseed meal varies with the extruder parameters: as the temperature of extruder sleeve rises, the tannin content first falls and then rises, without great fluctuation; as the rotation speed of extruder screw rises, the tannin content first rises and then falls; as the moisture of material rises, the tannin content first falls and then rises. Among the three factors selected in the test, the moisture of material is the major factor affecting the changes of tannin content, the rotation speed of extruder screw has the second influence, and the temperature of extruder sleeve in the selected range of 33~67°C has the least influence. The curve trend in dimension reduction analytic map basically corresponds with the conclusion of theoretical analysis.

Acknowledgements

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References

[1] Xu, L., Diosady, L.L., 1994.The production of Chinese rapeseed protein isolates by membrane process-ing. JAOCS, 71(3):935~939

[2] Pang,G.C.,Yan,Y.L.,Research on the Production of Compound Amino Acid by Rapeseed Meal. China Condiment, 1999 (8):18~23

[3]Jacques, G.,1990.Effect of Succinylation on Some Physicochemical and Functional Properties of the 12S Storage Protein from Rapeseed.Food Chem, 38(4):61~69

[4]Wang,Z.J., Jiang, Y.C., 2001.Preparation of N-acyl Peptide from Protease Hydrolysate of Rape Seed Cake (II)-Optimization of N-acyl Peptide Preparatio.Amino Acids & Biotic Resource, 23 (1): 29~31

[5]Wang,Z.J., Jiang, Y.C., 2001.Preparation of N-acyl Peptide from Protease Hydrolysate of Rape Seed Cake (II)-Optimization of N-acyl Peptide Preparatio.Amino Acids & Biotic Resource, 23 (2): 25~27

[6] Zhu, Z., 1995. Optimization of Processing Factors of Agricultural Product with Single-screw Extruder. Tran- sactions of the Chinese Society of Agricultural Engineering, 18(3):162~166

[7]Shen,D. C .,Meng, Y., Etc., 1999.Test Study on Extrusion Maize With Germ as Beer Adjunct.Transactions of the Chinese Society of Agricultural Engineering, 15 (2): 202~207

[8] Xu,Z.R.,The Optimum Regression Designs in Agricultural Experiments, Harbin: Heilongjiang Science and Technology Press, 1988

Identification of rapeseed oleosins, a family of emulsifying proteins, and optimization of their extraction from seeds and defatted meals using organic solvents

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Abstract

In oilseeds, lipids are stored in oil bodies, also called oleosomes. They are composed of a core of triacylglycerols surrounded by a monolayer of phospholipids in which different proteins are inserted. Eight proteins were previously identified in *Arabidopsis thaliana* oleosomes, the most abundant ones belonging to the oleosin family. These seed oleosins, named S1, S2, S3, S4 and S5, are amphiphatic proteins containing a long central hydrophobic domain highly conserved and two terminal hydrophilic domains, and therefore display emulsifying properties. In addition, oleosins are expected to be key factors for the stability of oleosomes and their study may contribute, in particular for rapeseed, to improve oil extraction using mild technologies while preserving proteins quality.

Here, we describe the oleosin content of *Brassica napus* oleosomes. These organelles have been purified by 6 flotation centrifugation steps in order to remove proteins non-specifically associated. Protein identification was carried out after SDS-PAGE separation, nano-LC-MS/MS analysis of trypsin peptides and comparison with *A. thaliana* protein database and *B. napus* EST databank. Major proteins were characterized as 15 different oleosins, representing up to 84% of oleosome proteins, and highly homologous to *A. thaliana* oleosins.

Specific antibodies raised against the N-terminal domain of *A. thaliana* oleosins, cross-reacted with rapeseed oleosins. Immunoblot analysis corroborated proteomic results, showing the presence of *B. napus* oleosins orthologous to *A. thaliana* S1, S2, S3, S4 and S5. These antibodies were used to set up a semi-quantitative assay of rapeseed oleosins by dot-blot. This assay allowed the study of oleosin extraction from seeds using organic solvents. One mixture of chloroform/methanol gave optimal extraction of all the oleosins. This protocol was also used on seed defatted meals and allowed the specific extraction of oleosins, identified by immunoblot and proteomic analyses. Surprisingly, oleosins extracted from seed defatted meals displayed the same apparent molecular mass than in seeds, showing that they are not degraded during industrial process. Together with oleosins, napins and cruciferins were also identified in this extract.

This is the first report showing the presence of oleosins in rapeseed defatted meals. A method for their specific extraction is described. The functional properties of this extract need to be further investigated to determine potential biotechnological applications.

Key words: Oleosins, *Brassica napus*, seed, oleosome, protein composition, organic solvent, extraction.

Introduction

Lipids required for energy supply during seedling growth are stored in oleosomes, also referred as oil bodies or lipid droplets. In exalbuminous oilseeds, like *Brassica napus* and *Arabidopsis thaliana*, these organelles are found in cotyledons and the embryonic axis. Oleosomes are often considered just as lipid balls because of their simple structure, but they display a remarkable stability, withstanding dessication, rehydratation and temperature variations. Understanding the molecular composition and organization of this organelle and its biogenesis should lead to identify key factors for its stability. This is a central question to answer in the aim to develop novel and milder technologies of rapeseed oil extraction, with improved efficiency and more preserved quality of protein by-products.

Oleosomes are small spheres with diameter ranging between 0.5 and 2 µm, depending of the specie. This size has been assumed to provide a high surface-to-volume ratio that would facilitate access by lipases during germination (1). They are composed of a core of neutral lipids (triacylglycerols) surrounded by a monolayer of phospholipids in which different proteins are embedded. The major proteins associated with oleosomes are oleosins, which are usually present as two or more isoforms. In *Arabidopsis thaliana*, 4 different isoforms of oleosins, named S1, S2, S3 and S4, are present and accounted for up to 79% of oil body proteins (2). Oleosins form a family with similar structural features that include a highly conserved core of hydrophobic residues flanked by hydrophilic or amphipathic N and C termini of various length. This central hydrophobic domain of ~70 amino acids is the longest hydrophobic fragment known to date and is believed to have an hairpin structure due to a conserved central proline knot (3). Protease protection assays revealed that oleosins are anchored in oil bodies by the hydrophobic domain, exposing the hydrophilic N- and C-terminal ends to the cytoplasm (4). Several indirect observations have suggested that oleosins promote electrical repulsion between oleosomes, preventing them from coalescing. Recently, the relation between oleosin content and oleosome size has been demonstrated using reverse genetic approach (5).

The interfacial properties of oleosins, suspected from their ability to be inserted to lipid particles *in vivo* and *in vitro*, were

analyzed on purified recombinant oleosins of *A. thaliana*. Oleosins decreased the interfacial tension at the oil/water interface more efficiently than β-casein, an emulsifying protein used in food industry (6). As oleosins represent up to 10% of the seed proteins in weight (7,8), they are a potential attractive source of natural emulsifier.

In the present work, we report the exhaustive description of the oleosin content of *Brassica napus* oleosomes. Moreover, we set up a simple protocol for specific extraction of oleosins from *B. napus* seeds and defatted meals. This procedure will be useful to investigate the potential use of oleosins as natural emulsifier.

Materials and methods

Oleosomes purification

Oleosomes were purified from mature seeds of *Brassica napus* hybrid Explus (generous gift of Monsanto, Saint-Louis, Missouri) as previously described (2).

Proteins identification

Proteins were separated by SDS-PAGE and stained with G-250 Coomassie blue. Protein bands were excised from the polyacrylamide gel and digested with trypsin. Trypsin digestion, peptide processing and nano-LC-MS/MS analysis of trypsin peptides were as previously described (2).

Immunoblot and dot-blot

For immunoblot analysis, proteins were resolved by SDS-PAGE before blotting on to PVDF membrane. For dot-blot, protein solubilized in organic solvents were spotted directly on to PVDF membrane. The membrane was probed with rabbit serum at various dilution: 1:5000 dilution for anti-S2 and anti-S3 sera, 1:4000 dilution for anti-S1 serum and 1:2000 for anti-S4 and anti-S5 sera. Peroxidase conjugated goat anti-rabbit IgG (Pierce) was used as secondary antibody. Saturation and incubation with antibodies were carried out for 90 min in TBS (20 mM Tris, 150 mM NaCl, pH 7.4) containing 0.05% Tween-20 and 5% skimmed dry milk. After each antibody incubation, the membrane was washed 3 times for 10 min in TBS containing 0.05% Tween-20. Peroxidase activity was revealed using SuperSignal West Dura Extended Duration Substrate (Pierce) according to the manufacturer protocol. Membrane was exposed to Kodak Biomax XAR film. Film was scanned (600 dpi) using an Epson Expression 1680 Pro scanner. The resulting TIFF file of dot-blot was analyzed using the Image Quant software (version 4.2a) from Molecular Dynamics (GE Healthcare).

Extraction of oleosins using chloroform/methanol

Mature seeds of *B. napus* hybrid Explus were grounded at 8 mg/mL using a glass Potter-Elvehjem grinder. Several grinding buffers were assayed: 50 mM Na carbonate pH 11, 10 and 9; 50 mM Tris-HCl pH 9, 8, 7 and 6; 50 mM Na citrate pH 5, 4 and 3. Extraction was performed at room temperature by slowly adding 9 volumes of chloroform/methanol mixture at various ratio: from 0/9 to 9/0. After mixing, the extract was centrifuged and the organic phase collected. For SDS-PAGE analysis, organic solvents were evaporated under a stream of N_2 and proteins were solubilized in non-reducing Laemmli buffer.

Protein measurement

Protein amounts in seeds, defatted meals and organic extracts were determined by amino acid measurement after alkaline hydrolysis, using bovine serum albumin as standard, as previously described (9).

Table I: Identified proteins in purified rape oleosomes.

Band	Relative Intensity (%)	Protein	Apparent Molecular Mass (kDa)	Sequence Molecular Mass (kDa)	Protein coverage (%)	pI	A thaliana orthologues
A	2.8	Contig 1048694	15	15.580	11.5	10.0	At5g51210/S5
B	71.3	Contig 1047406	18	20.645	19.3	8.3	At3g01570/S1
		Contig 1047407		20.576	27.1	8.3	At3g01570/S1
		Contig 1047290		19.871	24.0	9.2	At3g27670/S2
		Contig 1047291		19.748	34.2	7.1	At3g27670/S2
		Contig 1047291		18.913	25.8	9.1	At3g27670/S2
		Contig 1047292		20.117	40.0	7.8	At3g27670/S2
		Contig 1047157 (BnV)		20.434	20.5	9.1	At4g25140/S3
		Contig 1047158		19.870	22.5	9.3	At4g25140/S3
		Contig 1047159 (BnIII)		21.409	12.4	9.3	At4g25140/S3
		Contig 1047156 (NapII)		20.551	18.3	9.4	At4g25140/S3
		Contig 1047155		19.384	20.7	9.2	At4g25140/S3
		Contig 1047162		19.211	14.1	9.2	At4g25140/S3
C	10.1	Contig 1047654	22	22.893	47.5	9.1	At5g40420/S4
		Contig 1047657		22.952	45.2	8.8	At5g40420/S4
D	4.1	Contig 1047992	26	28.133	16.3	6.0	At4g26740/ caleosin
E	6.7	Contig 1048529	42	39.114	52.7	6.6	At5g50600/ steroléosin
F	5.0	β-glucosidase	62	56.283	10.1	6.0	At3g03640

Results and discussion

Oleosin content of rape oleosome

The proteins contained in the oleosomes from the last stage of purification were analysed by SDS-PAGE (figure 1). Upon gel scanning and image analysis, an approximate quantification based on band intensity was obtained (table I). Trypsin peptides obtained from the excised protein bands were analyzed with LC-MS/MS and protein identification was performed using databases not only of *Brassica napus* genome and ESTs but also of *Arabidopsis thaliana* genome. Results summarized in Table I show that oleosins are the major proteins of rape oleosomes, accounting for more than 84% of its protein content. The more intense band (figure 1, band B) contained 12 different oleosins with the same apparent molecular mass of 18 kDa. Comparison with *A. thaliana* oleosins showed that 6 of these rape oleosins were orthologues to S3, 4 to S2 and 2 to S1. Two more oleosins, homologous to *A. thaliana* oleosin S4, were identified in a second band, less intense, at a slightly higher apparent molecular mass (band C at 22 kDa). In addition, one faint band at 15 kDa (band A), accounting for 2.8 % of oleosome proteins, was shown to be the orthologue to *A. thaliana* oleosin S5. This is the first demonstration by proteomic analysis that oleosin S5 is present in oleosomes isolated from mature seeds.

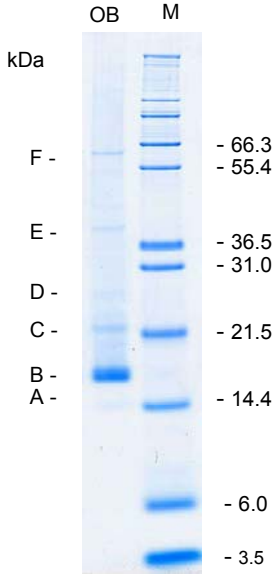

Figure 1: SDS-PAGE of proteins from purified rape oleosomes (OB). Protein bands are lettered as in Table I.

Cross-reactivity of antisera to A. thaliana oleosins with B. napus oleosins

Different sera were obtained by immunizing rabbits with bacterially expressed *A. thaliana* oleosins S1, S2, S3 and S5 (unpublished data). Only the N-terminal domain of each oleosin was used for immunization, as this portion is the less conserved among oleosins. Serum raised against *A. thaliana* oleosin S4 was described previously (6). Specificity of these 5 sera was demonstrated by immunoblot analysis of *A. thaliana* oleosins, either expressed in *E. coli* (unpublished data) or from purified oleosomes (figure 2). As these sera were shown to cross-react with *B. napus* oleosins (figure 2), they can be used to analyse rape oleosin content by immunoblot.

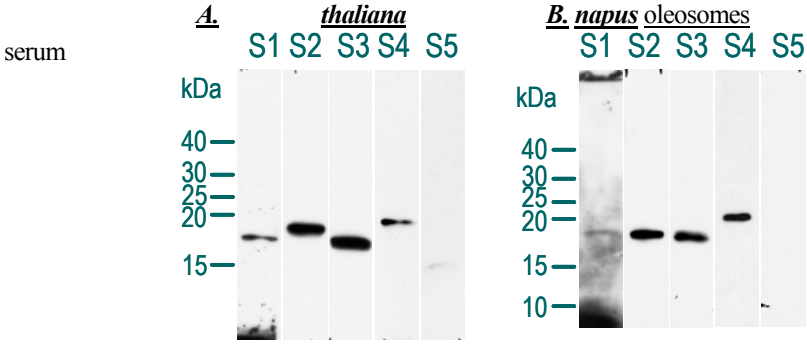

Figure 2: Specific sera to *A. thaliana* oleosins cross-reacted with proteins from purified rape oleosomes.

Optimization of oleosin extraction from seeds using organic solvents

The extraction protocol, adapted from (10), is based on the differential solubilization of hydrophobic proteins in chloroform/methanol mixture. The yield of rape oleosins solubilized in the organic phase was estimated using dot-blot assays with sera raised against oleosins S1, S2, S3 and S4 of *A. thaliana*. The pH effect of the grinding buffer was analyzed. The most

basic buffer gave the best extraction efficiency, whatever the oleosin. In a second step, seeds ground in 50 mM Na_2CO_3 (pH 11) were extracted with mixtures containing various chloroform/methanol ratios (from 0/9 to 9/0) in order to optimize the composition of the organic solvent. In monophasic mixtures (i.e. for chloroform/methanol ratios from 0/9 to 5/4), increasing chloroform content increased extraction efficiency, whatever the oleosin. In biphasic extracts (i.e. for chloroform/methanol ratios from 6/3 to 9/0), the organic phase was free of oleosins. In conclusion, the extraction was optimized as follow: seeds ground in 50 mM Na_2CO_3 and extracted with 9 volumes of chloroform/methanol 5/4. This protocol led to a specific enrichment in oleosins. Preliminary results indicated that oleosins represent around 50% of the proteins extracted from rape seeds.

Protein composition of rape seed and defatted meal extracts

This extraction protocol was applied to industrial rape defatted meals (Saipol, Grand-Couronne, France). The protein content of this extract was analyzed by SDS-PAGE and identified by immunoblot analysis, together with the seed extract one (figure 3). Both extracts contained all the 5 types of oleosin (S1, S2, S3, S4 and S5) in similar proportions to oleosomes, showing that extraction efficiency is within the same range whatever the oleosin. Proteomic analysis confirmed the presence of oleosins and identified contaminating proteins as napins and cruciferins.

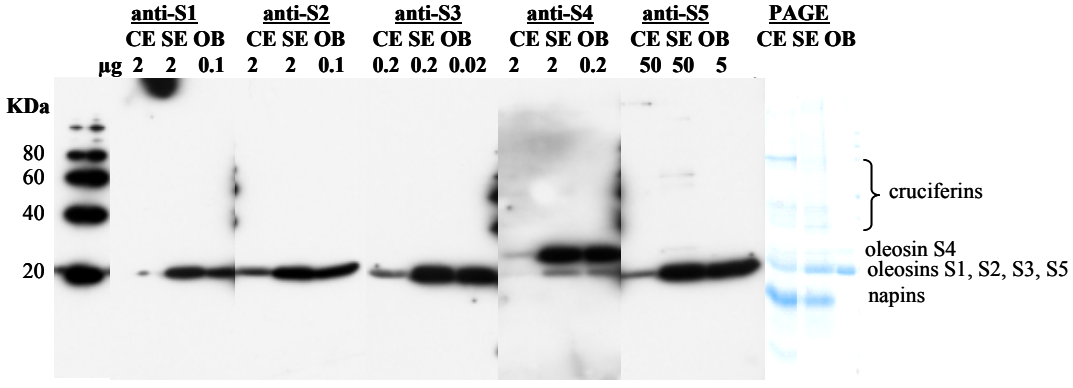

Figure 3: SDS-PAGE and immunoblot analyses of rape oleosomes (OB), seed extract (SE) and defatted meal extract (CE). Different amounts of oleosomes (µg of protein), seed extract (µg of extracted seeds) and defatted meal extract (µg of extracted defatted meals) were probed with serums against *A. thaliana* oleosins.

Conclusion

The protein complement of oleosomes purified from *Brassica napus* mature seeds was exhaustively analyzed, leading to the identification of 15 different oleosins.

As oleosins display original solubility properties, similar to lipid ones, they could be extracted using chloroform/methanol. In the present work, we described the optimization of a novel and rapid procedure for their specific extraction from seeds, without tedious oleosome purification. Moreover, the protocol was successfully applied to rape defatted meals, leading to an extract containing around 10-13% of the total proteins (preliminary results) and enriched in oleosins. In the future, interfacial properties of these seed and defatted meal extracts will have to be characterized.

Acknowledgements

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References

1. Tzen, J., Cao, Y., Laurent, P., Ratnayake, C., and Huang, A. (1993) *Plant Physiol* **101**, 267-276
2. Jolivet, P., Roux, E., D'Andrea, S., Davanture, M., Negroni, L., Zivy, M., and Chardot, T. (2004) *Plant Physiol. Biochem.* **42**, 501-509
3. Li, M., Murphy, D. J., Lee, K. H., Wilson, R., Smith, L. J., Clark, D. C., and Sung, J. Y. (2002) *J Biol Chem* **277**, 37888-37895
4. Tzen, J. T., Lie, G. C., and Huang, A. H. (1992) *J Biol Chem* **267**, 15626-15634
5. Siloto, R. M., Findlay, K., Lopez-Villalobos, A., Yeung, E. C., Nykiforuk, C. L., and Moloney, M. M. (2006) *Plant Cell* **18**, 1961-1974
6. Roux, E., Baumberger, S., Axelos, M. A., and Chardot, T. (2004) *J Agric Food Chem* **52**, 5245-5249
7. Huang, A. H. (1996) *Plant Physiol* **110**, 1055-1061
8. Murphy, D. J. (2001) *Prog Lipid Res* **40**, 325-438
9. Landry, J., and Delhaye, S. (1996) *Anal Biochem* **243**, 191-194
10. Seigneurin-Berny, D., Rolland, N., Garin, J., and Joyard, J. (1999) *Plant J* **19**, 217-228

FEED AND INDUSTRIAL RAW MATERIAL

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Feed

Chemical composition and nutritive value of yellow-seeded *Brassica napus* canola

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Abstract

Plant selection programs directed towards the development of yellow-seeded canola are justified as a means to improve meal quality without compromising oil content in the seed. The objective of this study was to compare a new yellow-seeded *B. napus* line YN01-429 with its black-seeded counterpart *B. napus* line N89-53, both types produced under identical growing conditions in two consecutive years (2003-2004). On average, in comparison to black-seeded, yellow-seeded type contained more oil (46.4 vs 43.1% DM) and less dietary fibre (14.4 vs 18.5%). When expressed on fat free basis, higher amounts of protein (48.6 vs 47.9%), and lower amounts of sucrose (7.5 vs 8.1%), oligosaccharides (2.1 vs 3.6%), starch (2.3 vs 2.8%), total phosphorous (1.14 vs 1.25%), non-phytate phosphorous (0.83 vs 0.93%), and glucosinolates (20.8 vs 27.4 μmol/g) were observed in yellow-seeded canola. Although slightly higher in the content of non-starch polysaccharides (17.5 vs 16.7%), the total dietary fibre averaged 26.4% for yellow- and 32.1% for black-seeded samples. Lower fibre content in yellow-seeded canola was reflected in lower content of lignin with associated polyphenols (4.7 vs 9.8%). When expressed in g/16g N, no major difference in essential amino acid contents was observed. In a two-week feeding trial, broiler chickens were fed corn (50%)/soybean meal (30%) diets containing 15% of ground seed from yellow- or black-seeded canola. The diets were formulated to contain 21% crude protein and 3050 kcal/kg available energy and were fed without or with exogenous enzyme supplementation. On average, chickens fed diets containing yellow seeds showed body weight gain (g/bird/14 days) and feed efficiency (g feed/g weight gain) values of 280.5 and 1.37 which were identical to those of 283.4 and 1.37 for the diets containing black seeds. Regardless of the seed coat color, weight gain and feed efficiency averaged 278.8 and 285.0, and 1.39 and 1.34 for the control and the enzyme supplemented diets, respectively.

Key words: Yellow-seeded canola, *Brassica napus*, chemical composition, nutritive value, broiler chickens, enzyme supplementation

Introduction

Research conducted to date has shown yellow-seeded *B. napus* canola to have superior quality characteristics to that of yellow-seeded *B. rapa*, *B. juncea* and the black-seeded type of *B. napus*, both in terms of chemical composition (i.e., lower fiber, lower phytate phosphorus, lower glucosinolate and higher protein content) and the overall nutritive value as determined with broiler chickens (Slominski, 1997; Jiang et al., 1999). A new and improved yellow-seeded *B. napus* canola has recently been developed at the Saskatoon Research Centre, Agriculture and Agri-Food Canada through crossing breeding of yellow-seeded lines, which in our earlier research were found to have superior quality characteristics.

The objective of this study was to compare a new yellow-seeded *B. napus* line YN01-429 with its black-seeded counterpart *B. napus* line N89-53, both types produced under identical growing conditions in two consecutive years.

Materials and Methods

The seed samples represented composite lines of near-isogenic yellow- and black-seeded *B. napus* canola grown in the field plots of the Agriculture and Agri-Food Canada research station in Saskatoon, Canada under identical growing conditions in years 2003 and 2004. Seed and laboratory prepared meals were subjected to oil, protein, amino acids, carbohydrates (sucrose, starch, oligosaccharides), glucosinolates, dietary fibre, total and non-phytate phosphorus analysis using the procedures described earlier (Slominski et al., 1994; Simbaya et al. 1995). The nutritive value of full fat seeds was investigated in a two-week broiler chicken trial. Chickens were fed corn (50%)/soybean meal (30%) diets containing 15% of ground seeds from either yellow- or black-seeded samples. The diets were formulated to contain 21% crude protein and 3050 kcal/kg available energy and were fed without or with exogenous enzyme supplementation. The enzyme supplement contained cellulase, pectinase, mannanase, xylanase, glucanase, galactanase and other enzyme activities. All chemical analysis and broiler chicken performance data were subjected to GLM procedure of SAS (SAS institute, 2003). Differences between means were determined by Tukey's test. The statements of significance are based on $P<0.05$.

Results and Discussion

Chemical composition of *Brassica* seed samples used in the study is shown Table 1. On average, in comparison with black-seeded, yellow-seeded type contained more oil (46.4% vs 43.1%), less protein (26.2 vs 27.2%) and less dietary fibre (14.4 vs 18.5%) with the crop year and seed coat color having significant effects on all three parameters measured. This is somewhat contradictory to our earlier research (Jiang et al., 1999) in which higher protein (25.0 vs 22.4%) and lower fat (44.9

vs 49.3%) contents were observed in yellow-seeded when compared with black-seeded samples. However, over the years more emphasis has been put on the improvement in oil content which resulted in the protein content being lower in the newly developed yellow-seeded line. Similar to our earlier research, however, the fiber content was found to be still significantly lower in this type of canola.

Table 1. Fat, protein and dietary fiber content of *B. napus* seeds of different color (% DM; full fat basis)

Seed type	Fat	Crude protein	Dietary fibre[1]
Black-2003	42.5[c]	29.1[a]	17.5[b]
Yellow-2003	43.7[b]	28.7[a]	14.6[c]
Black-2004	43.6[b]	25.3[b]	19.4[a]
Yellow-2004	49.0[a]	23.8[c]	14.1[c]
Pooled SEM	0.2	0.2	0.3
Year	<0.01	<0.01	0.02
Color	<0.01	<0.01	<0.01
Year × Color	<0.01	0.01	<0.01

[1] Includes non-starch polysaccharides, lignin with associated polyphenols, cell wall protein, and minerals; [a,b] Means within a column and within a source with no common superscript differ significantly (P<0.05).

When expressed on fat free basis, meals derived from yellow-seeded canola contained more protein, less starch and less sucrose (Table 2). The low sucrose content of yellow-seeded samples was different from our earlier data (Jiang et al., 1999) showing higher sucrose content in yellow- than black-seeded samples (i.e., 9.0 vs 7.8%). Such a discrepancy could be a consequence of breeding activities which may have influenced certain quality characteristics of the new line. Total and non-phytate phosphorus contents in yellow-seeded samples were slightly but significantly (P<0.05) lower than those present in the black-seeded samples. Lower contents of antinutritional factors, including glucosinolates and oligosaccharides raffinose and stachyose, were also observed in this new yellow-seeded canola.

Table 2. Chemical composition of meals derived from black- and yellow-seeded *B. napus* canola (% DM; fat free basis)

Seed type	Crude Protein	Sucrose	Oligo-saccharides[1]	Starch	Dietary fibre	Gluco-sinolates[2]	Total P	Non-phytate P
Black	47.9[b]	8.1[a]	3.6[a]	2.8[a]	32.4[a]	27.4[a]	1.25[a]	0.93[a]
Yellow	48.6[a]	7.5[b]	2.1[b]	2.3[b]	26.6[b]	20.8[b]	1.14[b]	0.83[b]
Pooled SEM	0.2	0.1	0.1	0.1	0.4	1.2	0.03	0.02

[1] Includes raffinose and stachyose; [2] μmol/g; [a,b] Means within a column with no common superscript differ significantly (P<0.05).

Although higher in the content of non-starch polysaccharides (17.5 vs 16.7 %), the total dietary fibre in yellow-seeded samples was found to be significantly lower, differing by 5.7 percentage points from that of the black-seeded canola (Table 3). Similar to our earlier research (Slominski at al., 1994; Simbaya et al., 1995, Jiang et al., 1999), lower fibre content of yellow-seed canola was associated with lower content of lignin and polyphenols. It could be hypothesized, that the relatively low degree of lignification and/or saturation of the seed coat cell walls with tannins (condensed polyphenols) could result in improved nutritive value of yellow-seeded canola. In addition, lower fibre content of the seed could potentially result in higher nutrient density of meals derived from the yellow-seeded type.

Table 3. Composition of dietary fibre in meals derived from black- and yellow-seeded *B. napus* canola (% DM)

Seed type	NSP[1]	Protein	Ash	Lignin and polyphenols	Total fibre
Black	16.7[b]	4.8	0.8	9.8[a]	32.1[a]
Yellow	17.5[a]	4.0	0.2	4.7[b]	26.4[b]
Pooled SEM	0.1	0.2	0.1	0.7	0.4

[1] Non-starch polysaccharides; [a,b] Means within a column with no common superscript differ significantly (P<0.05).

When expressed in g/16g N, no difference in essential amino acid content was observed except for arginine and valine levels which were slightly, but significantly lower in the yellow-seeded samples (Table 4).

Table 4. Selected amino acid content of *B. napus* canola of different color (g/16g N)

Color	Arginine	Histidine	Lysine	Methionine	Cystine	Threonine	Valine
Black	6.5[a]	2.7	6.2	2.1	2.4[b]	4.6	4.4[a]
Yellow	5.9[b]	2.7	6.2	2.2	2.6[a]	4.5	4.2[b]
Pooled SEM	0.1	0.1	0.1	0.05	0.04	0.1	0.02

[a,b] Means within a column with no common superscript differ significantly (P<0.05).

Chickens fed diets containing seeds of yellow color showed body weight gain and feed efficiency values similar to those fed diets containing black seeds (Table 5).

Table 5. Growth performance of broiler chickens (1-14 days of age) fed diets containing black and yellow *B. napus* seeds without and with enzyme supplementation

Year	Seed Type	Enzyme	Feed Intake (g/bird)	Body Weight Gain (g/bird)	Feed Efficiency
2003	Black	-	384.8 ± 9.6[1]	276.3 ± 9.9	1.40 ± 0.02[a]
		+	378.7 ± 9.6	286.4 ± 9.9	1.33 ± 0.02[b]
	Yellow	-	379.7 ± 10.1	273.1 ± 10.4	1.41 ± 0.02
		+	375.3 ± 9.6	275.9 ± 9.9	1.36 ± 0.02
2004	Black	-	386.7 ± 9.6	276.9 ± 9.9	1.40 ± 0.02
		+	394.6 ± 9.6	293.9 ± 9.9	1.34 ± 0.02
	Yellow	-	392.2 ± 10.1	289.1 ± 10.4	1.36 ± 0.02
		+	379.8 ± 9.6	284.0 ± 9.9	1.34 ± 0.02
Year[2]			NS	NS	NS
Color			NS	NS	NS
Enzyme			NS	NS	<0.01

[1]Mean±SD; [2]All the 2-way and 3-way interactions were tested non-significant; [a,b]Means within a column and within a source with no common superscript differ significantly ($P<0.05$).

Conclusions

Chemical characterization of canola seed samples of different color showed a new yellow-seeded *B. napus* line to contain more oil, less protein, less fibre, and less glucosinolates than its black-seeded counterpart. No differences in growth performance of broiler chickens fed diets containing 15% of either yellow or black seeds was noted. Carbohydrase enzyme addition had a positive effect on feed utilization with more pronounced effect observed for the diets containing black (high fibre) seeds.

References

Jiang P., Slominski B.A., Rakow G. 1999. Chemical composition and nutritive value of yellow-seeded *Brassica napus* canola for broiler chickens. Poult. Sci. **78,** 12.

Simbaya J., Slominski B.A., Rakow G., Campbell L.D., Downey R.K., Bell J.M. 1995. Quality characteristics of yellow-seeded *Brassica* seed meals: Protein, carbohydrates, and dietary fiber components. J. Agric. Food Chem. **43**, 2062-2066.

Slominski B.A. 1997. Developments in the breeding of low fibre rapeseed/canola. J. Anim. Feed Sci. **6**: 303-317.

Slominski B. A., Campbell L.D., Guenter W. 1994. Carbohydrates and dietary fiber components of yellow- and brown-seeded canola. J. Agric. Food Chem. **42**, 704-707.

Iodine in the milk – effects of iodine and rape seed feeds in the cow's diet and consequences for human nutrition

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Abstract

There is still iodine deficiency in many populations, which justifies efforts to increase the content of this trace element in food such as milk and eggs by fortifying the animal feed with extra iodine. Additionally to the amount of the trace element in the diet the milk-iodine concentration is affected by the occurrence of rape feeds. Former experiments with high and low glucosinolate rapeseed meal (RSM) in dairy cows (Papas et al., 1979) charakterized the glucosinolates as iodine antagonists, i.e. the milk iodine concentration was decreased by RSM feeding. Further dairy cow experiments with rapeseed feeds from 00 varieties either confirmed these findings (Emanuelson et al., 1993) or there was only a tendency of iodine milk concentration decrease and no effect on the total milk iodine amount in the milk amount per day (Jahreis et al.,1995).

The present study consisted of three parts. In the first part results of newer German studies of milk iodine concentration are presented. As second part, in a dose response experiment with lactating cows a broad range of iodine supplements was tested quantifying the effect of dietary iodine on the milk iodine concentration. As third part, the effect of a RSM with extremely low glucosinolate content should be tested on the milk iodine concentration.

Methods

The studies represented newer German investigations, as a rule in bulk milk from Thuringia and Bavaria.

In the dose-response experiment, five Holstein cows of the last lactation third were fed four iodine doses as calcium iodate-hexahydrate (with the mineral mixture, 150 g/d) in 4 periods of 14 days each. In addition to the testing of the diet without iodine supplement the supplementation levels were 15, 65 and132 mg iodine/cow/d corresponding to a total of 3, 17, 68 and 134 mg /day, i.e. 0.2 (basal diet), 1.3, 5.1 and 10.1 mg/kg dry matter (DM). Three milk samples per cow were taken during the last 5 days of each period; the daily milk yield amounted to 20 kg.

The RSM experiment consisted of two groups, with 24 Holstein-cows each, either without or with RSM (2 kg = 1.8 kg DM per animal and day) in the diet. Milk samples were taken from 9 cows of each group. The total mixed diets (Tab.1) contained in the control lupines as sole protein concentrate (18 % of the diet`s dry matter, DM), in the experimental group 11 % lupines + 8 % RSM (for details Kluth et al., 2005). The milk samples originated from the second experiment`s period – 12th. to 19th lactation week, 36 kg milk/ day (corrected for 4 % fat and 3.4 % protein), sampling after 5 weeks feeding of the respective diets.

The iodine analysis (Leiterer, 2001) was carried out applying the ICP-MS-technique (inductively coupled plasma mass-spectrometry) after a specific extraction of the freeze-dried milk with tetra methyl ammonium hydroxid (TMAH). The glucosinolate content of the RSM was analysed by HPLC (EU 1999, details by Rothe et al. 2004). The results are given as arithmetic mean and standard deviation The means were compared by the ANOVA and Dunnets test (dose response experiment) and by STUDENTs` t test (RSM experiment).

Results and discussion

Milk iodine concentration according to investigations of bulk milk samples

Due to the iodine supplementation of feed the milk iodine concentration increased from < 20 (Tab. 2) to > 100 µg/kg: Eastern Germany still during the beginning 1980s represents the example for a milk status without added iodine to the cow feed. Mean concentrations in the range of 100 to 200 µg iodine/kg milk show also further newer studies in Thuringia (Schöne et al. 2003, Bader et. al. 2005) and Saxony (Launer und Richter, 2005) with trends of decreased frequency of low iodine milk samples (<50 µg iodine/kg milk).

The German Society of Nutrition recommends a daily intake of 200 µg iodine for young people and adults (German Society of Nutrition… DACH 2000). One half litre of milk with 100 and 200 µg iodine/kg, resp., could contribute to the recommended amount at one quarter and one half, resp..

Milk iodine in dose response experiment

The milk iodine concentration did not differ between the sampling days (P=0.89 in the two way-ANOVA, not shown), however, the effect of dietary I dosage was significant. An about fourfold I supplementation in period 3 (65 mg I per day) as compared to period 2 (15 mg I per day) resulted in a 3.5 fold milk I concentration. A further doubling of the daily I dosage (132 mg I/day in period 3 versus 65 mg I/day in period 4) resulted in a further2.3 fold milk I concentration.

Relating the total iodine output represented by 20 kg milk/day to the ingested trace element, there was a transfer into the milk in a magnitude of 30 - 40 % in the three groups with the iodine supplements. In the period of no additional dietary iodine (only basal content), the transfer of three quarter related to the intake seems to be an artefact because the iodide resulting from the degradation of thyroid hormones would be effectively recycled (Schöne et al. 2001) and so it contributes to the milk iodine output in case of a dietary iodine deficit.

Effects of glucosinolates of RSM on the milk iodine concentration

The RSM contained 5,9 mmol glucosinolates/kg. The mineral mixture was labelled with 100 mg iodine /kg; one half year after the experiment only 59,6 mg iodine /kg were determined. Consuming as mean 230 g per day the mean daily iodine amount per cow via the mineral mixture with labelled content amounted to 23 mg iodine, i.e. 1 mg added iodine/kg DM with 23 kg DM as mean daily intake. Such dietary iodine represents the twofold concentration of 0. 5 mg iodine/ kg DM recommended by the GfE (2001).

Administering the RSM (0.4 mmol GSL/kg feed DM) significantly lowered the milk iodine concentration ($P < 0.001$), by about one half (162 ± 38 µg/kg milk) compared to the control group (356 ± 39 µg iodine/kg milk) with the diet free of rape feeds and glucosinolates, resp. (Fig.2). Inspite this milk iodine loss a concentration of significantly more than 100 µg iodine /kg milk also in the group with RSM points to adequate supply with the trace element.

Conclusion

In the dose-response experiment, the highest iodine feed supplement tested resulted in a milk iodine concentration exceeding the upper limit for human iodine consumption of 500 µg/d (German Society of Nutrition 2000) in case of the consuming only 250 ml of this fortified milk. As a result of this cow experiment, in the European Union the prevailing maximum iodine level of cow and hen diets was reduced from 10 mg/kg feed to 5 mg /kg feed (basis 880 g dry matter/kg diet). A cow experiment with solvent extracted RSM was presented in which already low amounts of glucosinolates decreased the milk iodine concentration. These effects of rape feeds confirm a glucosinolate iodine antagonism - however, in a new context of a possibly too high milk iodine concentration this effect is not *a priori* negative and contrasts with the traditional view of the glucosinolates in animal nutrition.

Table 1: Composition of the diets without and with rapeseed meal in the experiment with 24 cows per group. In both the groups the feed consumption amounted to 23 kg dry matter,DM /d.

Rapeseed meal	Without	With
Ingredients (% of the DM)		
grass silage, 1st cut	15.9	15.9
maize silage	39.3	38.6
straw	1.6	1.5
ensiled distillers grains	6.1	5.8
barley, crushed	15.7	15.9
lupines	18.3	10.9
rapeseed meal solvent extracted	-	8.4
mineral premix	1.0	1.0
rest[1]	2.1	2.0
Feed data		
NEL, MJ/kg DM	7.4	7.3
uCP g/kg DM	166	171
crude fiber g/kg DM	157	154

DM - dry matter, NEL – net energy lactation, uCP – (duodenally) utilizable protein (GfE, 2001) [1] propylene glycol, protected fat, lime stone, salt

Table 2: Studies in the iodine concentration of milk. In German Food Tables (Souci et al., 2000) 27 µg iodine/kg are given.

State, year	Number	0 ± s (Min. – Max.) Iodine µg/kg	Literature
Eastern Germany Before 1985[1)]	No numbers	17 ± 10	Anke et al. 1993
1987[2)]		53 ± 35	
1990[2)]		81 ± 11	
Bavaria 1995	368	114 (26 – 298)	Preiss et al. 1997
Thuringia 1996	61	111 ± 71 (15 –290)	Jahreis et al. 1999

[1)] before [2)] after the obligatory iodine addition to the mineral premixtures (cited in Flachowsky et al., 2006)

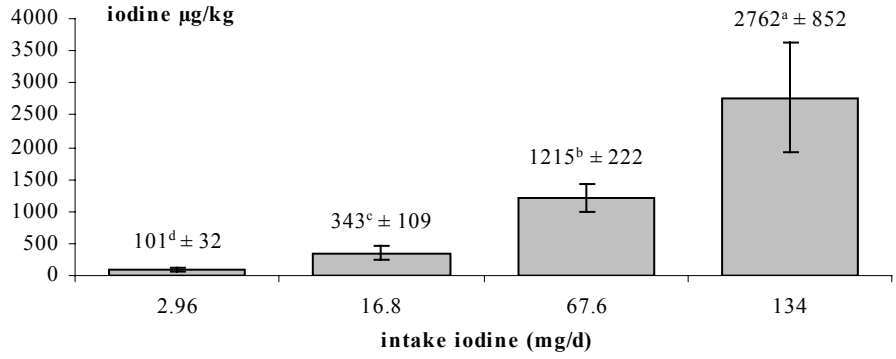

Fig.1: Exp 1 dose-response – milk iodine concentration, mean ± standard deviation (5 cows x 3 day samples). The supplementation was made exclusively by the mineral feed, whereof 150 g/day were offered. In the mineral feed without supplementation 6mg iodine/kg were detected, the further components (maize and grass silage contributed 2 mg iodine per day.

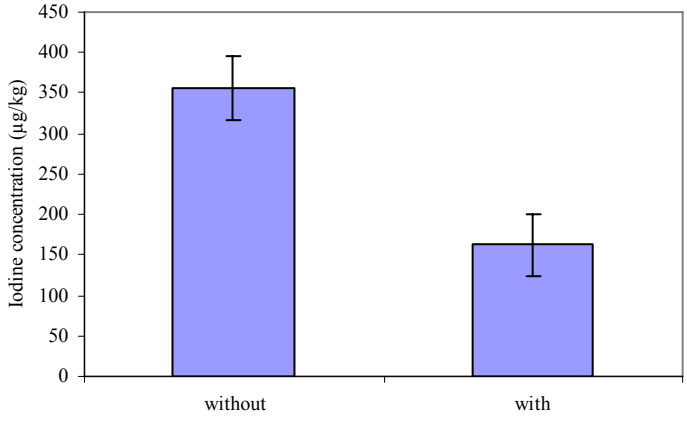

Fig. 2: Iodine concentration of the milk after 5 weeks feeding diets without or with rapeseed meal (mean ± standard deviation), 9 milk samples/group, significant difference by the t-test (P<0.001)

References

Bader, N.; U. Moller, M. Leiterer, K. Franke, G. Jahreis (2005): Tendency of increasing iodine content in human milk and cow's milk. Exp. Clin. Endocrinol. Diabetes 113, 8-12

Emanuelson, M.; K.- A. Ahlin, H. Wiktorsson (1993): Long-term feeding of rapeseed meal and full-fat rapeseed of double low cultivars to dairy cows. Livestock Production Science 33, 199 – 214

European Community (1990): Oilseeds - determination of glucosinolates - high performance liquid chromatography. *Official Journal of European Commission*. L 170, 27-34

Flachowsky, G.; F. Schöne, G. Jahreis (2006): Zur Jodanreicherung in Lebensmitteln tierischer Herkunft. Ernährungsumschau Ernährungs-Umschau 53, 17-21

German Society of Nutrition, Deutsche Gesellschaft für Ernährung....., D.A.CH.: Referenzwerte für die Nährstoffzufuhr. Umschau Braus GmbH, Verlagsgesellschaft, Frankfurt/M., 1. Aufl. (2000)

GfE - Gesellschaft für Ernährungsphysiologie der Haustiere (2001): Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlags GmbH Frankfurt am Main, 136 Seiten

Jahreis, G.; M. Leiterer, K. Franke, W. Maichrowitz, F. Schöne, V. Hesse (1999): Jodversorgung bei Schulkindern und zum Jodgehalt der Milch. Kinderärztl. Praxis 16 172-181

Jahreis, G.; G. H. Richter, H. Hartung, G. Flachowsky, F. Lübbe (1995): Einsatz von Rapskuchen in der Milchviehfütterung und Einfluß auf die Milchqualität. Das Wirtschaftseigenen Futter 41, 99- 114

Kluth, H. ; J. Boguhn, T. Engelhard, M. Bulang, M. Rodehutscord (2006): Bewertung von thermisch behandelten Lupinen als Rationskomponente für Hochleistungskühe. Bericht ufop Projekt, 25 Seiten

Launer, P.; O. Richter (2005): Untersuchungen zur Iodkonzentration im Blutserum von Milchkühen aus Sachsen sowie in Kuhmilch und Milchprodukten (Säuglingsnahrung). Berl. Münch. Tierärztl. Wochenschr. 118, 502-508

Leiterer, M.; D. Truckenbrodt, K. Franke (2001): Determination of iodine species in milk using ion chromatographic separation and ICP-MS detection. European Food Research Technology 213 150 – 153

Papas, A.; J.R. Ingalls, L.D. Campbell (1979): Studies on the effects of rapeseed meal on thyroid status of cattle, glucosinolate and iodine content of milk and other parameters. J. Nutr. 109, 1129 - 1139

Preiss, U.; C. Alfaro Santos, A. Spitzer, P. R. Wallnöfer(1997): Iodine content of Bavarian consumer milk. Z. Ernährungswiss. 36 220-224

Rothe, R., H. Hartung, G. Marks, H. Bergmann, R. Götz, F. Schöne (2004): Glucosinolate content in vegetative tissues of winter rape cultivars. Journal of Applied Botany 78, 41 – 47.

Schöne, F., Leiterer, M., Hartung, H., Jahreis, G., Tischendorf, F. (2001): Rapeseed glucosinolates and iodine in sows affects the milk iodine concentration and the iodine status of piglets. British Journal of Nutrition 85, 659 – 670.

Schöne, F.; M. Leiterer, H. Hartung, C. Kinast, A. Greiling, V. Böhm, G. Jahreis (2003): Trace elements and further nutrition-related constituents of milk and

cheese. Milchwissenschaft 58, 486-490
Souci, F.W.; W. Fachmann, H. Kraut.: Food Consumption and Nutrition Tables, 6th rev. edn. Medpharm, Scientific Publishers, Stuttgart, Germany(2000)

Effects of xylanase supplementation on digestibility and performance of growing-finishing pigs fed Chinese double-low rapeseed meal inclusion diets: in vitro and in vivo studies

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Abstract

An in vitro and a feeding trial were conducted to investigate the effect of xylanase supplementation on the feeding value of growing-finishing pig diets containing high proportions of Chinese double-low rapeseed meal (DLRM). Seven diets were formulated to meet NRC (1998) nutrient requirements. Diet 1 based on corn and soybean meal was used as positive control 1, and diet 2, a practical diet incorporated with conventional level of Chinese DLRM (6% and 10% of diet for the growing and finishing phase, respectively), as positive control 2. Diet 3 contained higher level of DLRM (10% and 15% of diet for the growing and finishing phase, respectively) as the negative control. Diet 3 plus xylanase at 0.10, 0.25, 0.50 and 0.70 g/kg diet created diets 4, 5, 6 and 7, respectively. The seven diets for the growing phase with triplicate each were incubated by the in vitro two-stage incubation method and digestibility of DM, CP and NDF was determined. In vitro, the negative control had a lower CP ($P < 0.05$) and NDF ($P = 0.06$) digestibility than positive control 1. Both DM and CP digestibility were increased ($P < 0.05$) by xylanase supplementation either at 0.50 or 0.70 g/kg diet, and NDF digestibility was improved following any test levels of xylanase addition. There was a high linear correlation ($r2 > 0.90$, $P < 0.05$) between the activity of the enzyme when transformed into its logarithmic value and in vitro digestibility of DM, CP or NDF. In the feeding trial, 112 crossbred pigs were randomly assigned to seven dietary treatments with 16 replicate pens of one pig each. Within the inclusion levels of xylanase an obvious dose effect on growth rate was observed ($r2 = 0.79$, $P < 0.05$) and xylanase addition at 0.70 g/kg diet resulted in significantly increased ADG over the negative control (878 g/d vs. 828 g/d, $P < 0.05$) during the growing phase, whereas the similar inclusion effect was not observed during the finishing phase. It would appear that nutritional values of corn and Chinese DLRM diet by pigs could be enhanced and therefore it was feasible to improve dietary inclusion levels of Chinese DLRM by appropriate amount of xylanase addition.

Key words: Chinese DLRM, Digestibility, Performance, Pigs, Xylanase

Introduction

There has been a considerable interest in using double-low rapeseed meal (DLRM) as a replacement of soybean meal in monogastric animal diets. However, the unrestricted use of this feedstuff in rapidly growing animals is limited by low available energy resulting from high level of non-starch polysaccharides (NSP) in the cell wall component (Simbaya et al., 1996). Our previous study has clearly indicated a significant decrease in weight gain when the inclusion of Chinese DLRM is higher than 100 g/kg diet in growing-finishing pig diets (Peng et al., 1995). More recently, Chinese DLRM was considered to be inferior to Canadian canola meal owing to the higher content of neutral detergent fibre (NDF) (306.6 g/kg vs. 215.4 g/kg, Chen et al., 2006).

It was reported that the levels of starch, free sugars and soluble NSP in DLRM is about 150 g/kg, which should contribute to a considerable digestible energy (Slominski and Campbell, 1990). Unfortunately, it appears that these carbohydrates are encapsulated by cell walls and that their actual contribution to digestible energy is modest (Bell, 1993). In this regard, it may be quite promising that using fibre-degrading enzymes to disrupt cell walls thus release entrapped nutrients and improve nutrient utilization of Chinese DLRM-containing diets. Previous evidence has demonstrated the effectiveness of xylanase supplement in improving the growth performance of broilers (Bedford and Morgan, 1995) and growing pigs (Fang et al., 2006) fed DLRM inclusion diets. To our knowledge, however, few study reports are available that investigate the feasibility of using xylanase preparation to improve the feeding value of growing-finishing pig diets containing higher proportions of DLRM, which is normally incorporated with less than 50 ~ 60 g/kg diet in the growing phase, whereas no more than 100 g/kg diet is recommended in the finishing phase (Peng et al., 1995).

In the present study, one of our aims was to examine whether it was effective to improve the Chinese DLRM inclusion levels in pig diets by xylanase supplementation. At the same time, attempts were made to find an appropriate dosage of xylanase addition considering that enzyme concentrations would also be an important determinants of the extent of cell wall hydrolysis (Tervilai-Wilo et al., 1996) and growth improvement (Zhang et al., 1996; Fang et al., 2006).

Materials and methods

Basal diets and treatments: Three basal diets were formulated to meet NRC (1998) nutrient requirements containing the

same level of calcium, phosphorus, amino acids and other necessary micro-components. Diet 1 based on corn and soybean meal (CSM) was used as positive control 1, and diet 2, a practical diet incorporated with conventional level of Chinese DLRM (up to 60 and 100 g/kg diet for the growing and finishing phase, respectively), the incorporation rate of which has been justified by Peng et al. (1995), was used as positive control 2. Diet 3 contained higher level of DLRM (up to 100 and 150 g/kg diet for the growing and finishing phase, respectively) as the negative control. Diet 3 plus xylanase at 0.10, 0.25, 0.50 and 0.70 g/kg diet created diets 4, 5, 6 and 7, respectively. The xylanase preparation were provided by Finnfeeds International Pte Ltd and contained endo-1,4 beta-xylanase (No EC 3.2.1.8.) 8,000 U/g, fermented from *Trichoderma Longibrachiatum*. All diets were in mash form. The basal diet formulations were presented in Table 1.

Table 1. The formulation of the three two-phase basal diets (g/kg)[a]

Ingredients	Growing phase			Finishing phase		
	1	2	3	1	2	3
Corn	690	635	595	730	640	590
Chinese double-low rapeseed meal	-	60	100	-	100	150
Wheat bran	-	60	100	-	90	145
Fishmeal	20	20	20	10	-	-
Soybean meal	250	185	145	220	130	75
Premix[a,b]	40	40	40	40	40	40
Enzyme A	-	-	-	-	-	-
Enzyme B	-	-	-	-	-	-
Nutrients as calculation						
DE (MJ/kg)	14.09	13.63	13.33	14.09	13.54	13.17
Crude Protein	180.0	179.0	180.0	163.0	164.0	163.0
Calcium	8.1	8.2	8.2	7.0	7.8	7.9
Total phosphorus	6.5	7.2	7.5	5.6	6.3	6.7
Available Phosphorus	3.3	3.3	3.3	2.6	2.6	2.6
Lysine	10.9	11.3	11.9	8.6	8.0	8.2
Apparent digestible Lysine	8.2	8.4	8.8	6.5	6.1	6.1
Apparent digestible methionine +cystine	4.7	4.9	5.1	4.5	5.0	5.2

[a]Provided per kg of diet for the growing and finishing (in brackets) phase : Vitamin A, 7200 (4950) IU; Vitamin D3, 1600 (1100) IU; Vitamin E, 12.8 (8.8) mg; menadione, 1.6 (1.1) mg; thiamine, 1.6 (1.1) mg; riboflavin, 4 (2.75) mg; niacin, 16 (11) mg; d-panthothenic acid, 8 (5.5) mg; Vitamin B6, 1.6 (1.1) mg; Vitamin B12, 12 (8.25) µg; d-biotin, 64 (44) µg; folic acid, 0.8 (0.55) mg. copper, 250 (125) mg; iron, 140 (140) mg; manganese, 50 (40) mg; zinc, 200 (160) mg; iodine, 0.8 (0.8) mg; selenium, 0.4 (0.3) mg. flavours, 120 (80) mg; antioxidant, 120 (120) mg.

In vitro two-stage enzyme incubation trial: The seven diets were incubated in triplicate with the *in vitro* two-stage enzyme incubation and dialysis procedure as described in detail by Peng (2000). The residues from the dialysis tubes were then frozen, freeze-dried and analyzed for DM and CP using the technique outlined by AOAC (1990). NDF content in diets and residue was determined by the method of Goering and Van Soest (1970). Each sample was analyzed in duplicate and the *in vitro* digestible DM, CP and NDF were calculated by subtracting the amount of DM, CP and NDF remaining in the residue from the present in the original diet. The digestibility coefficients were calculated from the following equation (taken CP as an example): CP digestibility coefficient = digestible CP (g/kg diet)/total dietary CP (g/kg diet)

Feeding trial: A total of 112 healthy crossbred pigs (average initial BW of 22.5 kg) were allotted, based on weight and sex, to seven dietary treatments with 16 replicate pens of one pig each. All pigs were housed in the same piggery and the total experimental period involving the growing phase (2 ~ 4 Months of age) and the finishing phase (4 ~ 5 Months of age) lasted 80 days. Pigs were fed thrice and twice per day during the growing and finishing phase, respectively. Pigs were fed *at libitum* and had free access to water. Pigs were weighed individually at the beginning and the end of each phase, and feed intake was recorded daily for each pen. Average daily gain (ADG) and the feed to gain ratio (feed : gain) were calculated from these data.

Statistical analysis: The study was conducted in a randomized complete block design. Data from the in vitro and feeding trial were statistically analyzed using one-way ANOVA procedure of the SAS statistical package (SAS, 1989). The means of the data from the in vitro trial and the performance trial where appropriate were also subjected to regression analysis using linear polynomials where the enzyme activity was transformed into its logarithmic value.

Results and discussion

Effects of DLRM inclusion levels and enzyme addition on in vitro digestibility

Effects of enzyme addition on in vitro digestibility of DM, CP and NDF of diets containing Chinese DLRM were presented in Table 2. The negative control had a lower CP ($P < 0.05$) and NDF ($P = 0.06$) digestibility than positive control 1, indicating the remarkable negative effect of the higher fibre level resulting from Chinese DLRM inclusion on nutrient utilization. Both DM and CP digestibility were increased ($P < 0.05$) by xylanase supplementation either at 0.50 or 0.70 g/kg diet, and NDF digestibility was improved following any test levels of xylanase addition. The improved digestibility indicated the positive effect of NSP-degrading enzyme preparation in enhancing the nutritive value of Chinese DLRM-containing diets. The increased NDF digestibility revealed that the improved nutrient digestibility was associated with the degradation of

dietary fibres (Slominski and Campbell, 1990; Fang et al., 2006).

Table 2. Effects of enzyme addition on in vitro DM, CP and NDF digestibility (%)

Parameter	Treatments[e]							S.E.M.
	1	2	3	4	5	6	7	
DM[f]	0.46[ab]	0.43[b]	0.43[b]	0.46[ab]	0.46[ab]	0.48[a]	0.48[a]	0.013
CP[f]	0.70[a]	0.61[d]	0.61[d]	0.65[c]	0.67[bc]	0.69[ab]	0.69[ab]	0.009
NDF[f]	0.12c	0.11c	0.09c	0.18b	0.18b	0.22a	0.20ab	0.010

[e]Treatment 1: control diet 1 based on corn and soybean meal; Treatment 2: control diet 2 containing Chinese double-low rapeseed meal (DLRM) at 60 g per kg of the total diet; Treatment 3: negative control containing Chinese DLRM at 100 g per kg of the total diet; Treatment 4 to 7: the same as treatment 3 except supplementation with enzyme B, respectively, at 0.10, 0.25, 0.50 and 0.70 g/kg diet, and the resulted xylanase activity were 0, 800, 2000, 4000, and 5600 U/kg diet, respectively.

[f]Means (expressed as the mean value of 3 replicates) within the same row with no common letters differ ($P < 0.05$)

The regression analysis results for in vitro digestibility of DM, CP and NDF were shown in Fig. 1. In the regression analysis, the enzyme activity value that was used for the diet with no enzyme supplementation was 3.27 U/kg diet, and this value was calculated according to the method described in detail by Zhang et al. (1996). As shown in Figure 1, DM, CP and NDF digestibility were all increased with the increase of enzyme concentration, and there was a high linear correlation ($r^2 > 0.90$, $P < 0.05$) between the activity concentration (X) of the enzyme when transformed into its logarithmic value and in vitro digestibility coefficients (Y) of DM, CP or NDF. The general equation could be presented as $Y = A + \log X$ where Y = in vitro digestibility coefficients of DM, CP or NDF, A = the intercept (y axis), B = the slope of the line, and X = the enzyme activity value (units per kg diet). The dose response suggested that the enhancement of nutrient digestibility was largely related to the active concentrations of NSP enzymes towards their specific substrate. It is obvious from this relationship that relatively small amounts of enzyme can have a dramatic effect on digestibility, whereas much larger amounts are required for each additional incremental improvement (Zhang et al. 1996).

Effects of DLRM and enzyme inclusion levels on pig performance

In the current study, the diet containing 60 and 100 g of Chinese DLRM/kg diet for the growing and finishing phase, respectively, was one that commonly used in commercial practice. Under the recommended inclusion levels of Chinese DLRM, no difference in performance was observed between the conventional CSM diet and the practical diet with the inclusion of Chinese DLRM at 60 ~ 100 g/kg diet (Table 3), although the latter had a lower DE (13.63 vs. 14.09 MJ/kg, Table 1) than the former as a result of decreased nutrient digestibility. This may be associated with that a diet with more balanced amino acid profile could be obtained when soybean meal and DLRM are in combination use rather than single soybean meal is used as a protein source in corn based diet (Peng et al., 1995). However, the negative effect caused by high dietary fibre may outweigh the positive effect arisen from the tendency towards more balanced amino acid profile when higher proportions of DLRM are incorporated into diet. This was supported by the decreased growth rate in Month 2 ~ 4 of pigs fed diet containing 100 g of Chinese DLRM/kg diet with dietary DE (14.09 vs. 13.33 MJ/kg, Table 3) 5% lower than conventional CSM diet. The increased growth rate following xylanase supplementation in the negative control provided further evidence for the negative effect resulting from dietary fibre.

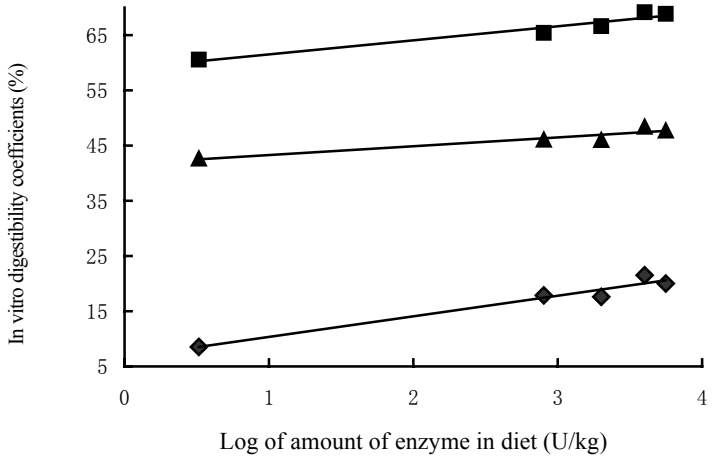

Fig.1. The linear relationship between in vitro digestibility and the amounts of enzyme transformed into their logarithmic values as determined from the equation $Y = 0.417 + 0.016 \log X$ ($r^2 = 0.90$, $P < 0.05$), $Y = 0.590 + 0.025 \log X$ ($r^2 = 0.94$, $P < 0.01$), or $Y = 0.066 + 0.037 \log X$ ($r^2 = 0.96$, $P < 0.01$) where X = units of enzyme in the diet and Y = in vitro digestibility coefficients of DM, CP, or NDF, respectively. Mean experiment values for in vitro digestibility of DM (▲), CP (■) and NDF (◆) were shown in Table 2. The activity value that was used for the diet with no enzyme supplementation was 3.27 U/kg diet. See text for the derivation of this value.

Table 3. Effects of enzyme addition on the performance of pigs fed Chinese double-low rapeseed meal (DLRM) containing diets for the growing and finishing phase

Treatments[d]	1	2	3	4	5	6	7	S.E.M
Weight, kg								
Month 2	22.7	22.4	22.5	22.6	22.5	22.4	22.5	0.49
Month 4	69.6	68.8	68.0	69.2	69.3	70.1	70.8	1.05
Month 5	90.1	91.0	89.9	90.3	89.2	91.8	91.2	1.38
ADG[e], g								
Months 2 ~ 4	853ab	844ab	828b	848ab	851ab	868ab	878a	15.0
Months 4 ~ 5	807bc	889a	874a	844abc	787c	855ab	790c	24.3
Months 2 ~ 5	843	858	843	844	835	869	854	15.3
ADFI[e], g								
Months 2 ~ 4	2088	2058	2075	2089	2080	2136	2089	45.9
Months 4 ~ 5	2610	2580	2602	2453	2534	2661	2598	74.8
Months 2 ~ 5	2266	2221	2239	2204	2230	2306	2251	50.5
Feed : gain[e]								
Months 2 ~ 4	2.45	2.44	2.50	2.47	2.45	2.46	2.39	0.046
Months 4 ~ 5	3.25a	2.92b	2.98b	2.91b	3.23a	3.11ab	3.32a	0.074
Months 2 ~ 5	2.69	2.59	2.66	2.62	2.67	2.66	2.64	0.043

[d]Treatment 1: control diet 1 based on corn and soybean meal; Treatment 2: control diet 2 containing Chinese DLRM at 60 g (growing phase) or 100 g (finishing phase) per kg of the total diet; Treatment 3: negative control containing Chinese DLRM at 100 g (growing phase) or 150 g (finishing phase) per kg of the total diet; Treatment 4 to 7: the same as treatment 3 except supplementation with enzyme B, respectively, at 0.10, 0.25, 0.50 and 0.70 g/kg diet, and the resulted xylanase activity were 0, 800, 2000, 4000, and 5600 U/kg diet, respectively.

[e]Means within the same row with no common letters differ ($P < 0.05$).

Within the inclusion levels of xylanase an obvious dose effect on growth rate was observed ($r2 = 0.79$, $P < 0.05$) and xylanase addition at 0.70 g/kg diet resulted in significantly increased ADG over the negative control (878 g/d vs. 828 g/d, $P < 0.05$) during the growing phase. The results revealed that a desirable performance could be obtained on condition that the supplemented enzyme had adequate activities towards its target substrates. A similar conclusion has been obtained by Zhang et al. (1996). These results indicate that it is necessary for the determination of appropriate dosage of enzymes when they are used to specific animal feeds. The negative control supplemented with xylanase at 0.5 or 0.7 g/kg diet had a similar even better growth performance compared with the positive control demonstrated the feasibility to increase the Chinese DLRM inclusion levels in swine diets by appropriate concentration of xylanase addition. Notably, the similar enyme inclusion effect was not observed during the finishing phase. The difference in response of pigs over different weight range may be associated with the difference in the development of gastrointestinal tract (Feng and Wang, 2004). In general, young animals are less mature in their digestive organ and, consequently, are more susceptible to the anti-nutritional effects of NSP present in diets. However, compared with the negative control, the similar feed efficiency but numerically higher growth rate (11 ~ 26 g/d) following xylanase addition at 0.5 or 0.70 g/kg diet still implied the positive effect of enzyme supplementation in Chinese DLRM-containing diets on pig performance during the overall phase.

Conclusion

The results indicated that it was feasible to improve the inclusion levels of Chinese DLRM in growing-finishing pig diets by appropriate amount of xylanase supplementation, and that in vitro two-stage enzyme incubation method could be used to predict the responses of pigs to exogenous enzymes and hence select effective enzymes targeting specific substrates.

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References

AOAC. (1990). Association of Official Analytical Chemists. Official Methods of Analysis; S. Williams, ed. AOAC: Arlington, VA.

Bedford M.R. Morgan A.J. (1995). The use of enzymes in canola–based diets. In: W. van Hartingsveldt, M. Hessing, J. P. van der Lugt, W. A. C. Somers (eds), 2nd European Symposium on feed enzymes. Proceedings of ESFE2, Noordwijkerhout, The Netherlands, 125-131.

Bell J. M. 1993. Factors affecting the nutritional value of canola meals: A review. Canadian Journal of Animal Science **73**, 679-697.

Chen G., Peng J., Liu Z.L., Fang Z.F. (2006). Evaluation of quality characteristics and its influencing factors of Chinese rapeseed cakes and rapeseed meals. Journal of the Chinese Cereals and Oils Association. **26**, 95-99.

Fang Z.F., Peng J., Liu Z.L., Liu Y.G. (2006). Responses of non-starch polysaccharide-degrading enzymes on digestibility and performance of growing pigs fed a diet based on corn, soybean meal and Chinese double-low rapeseed meal. Journal of Animal Physiology and Animal Nutrition. In press.

Feng D.Y., Wang, J. (2004). Advances in studies on the mechanism and influencing factors of non-starch polysaccharide enzyme preparations utilized in animal nutrition. Pages 317-324 in Advances in Animal Nutrition. D. F. Li, ed. Beijing, China.

NRC. (1998). Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.

Peng J. (1995). The experiment of substituting Chinese canola meal for soybean meal on equal nitrogen basis in diets for growing-finishing pigs. China Feed. (24), 13-16.

Peng J. (2000). Evaluation and improvement of quality of Chinese double-low rapeseed meal. Ph.D. Diss., Huazhong Agric. Univ., Wuhan, Hubei.
SAS Institute Inc. 1989. SAS/STAT User's Guide: Version 6. 4th edn. SAS Institute Inc., Cary, North Carolina.
Simbaya J., Slominski B.A., Guenter W., Morgan A., Campbell L.D. (1996). The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: In vitro and in vivo studies. Anim. Feed Sci. Technol **61**, 219-234.
Slominski B.A., Campbell L.D. (1990). Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. Journal of the Science of Food and Agriculture **53**, 175-184.
Tervila-Wilo A., Parkkonen T., Morgan A., Hopeakoski-Nurminen M., Poutanen K., Heikkinen P., Autio K. (1996). In vitro digestion of wheat microstructure with xylanase and cellulose from Trichoderma reesei. Journal of Cereal Science **24**, 215-225.
Zhang Z., Marquardt R.R., Wang G., Guenter W., Crow G.H., Han Z., Bedford M.R. (1996). A simple model for predicting the response of chicks to dietary enzyme supplementation. Jornal of Animal Science **74**, 394-402.

Nutritional and anti-nutritional composition of rapeseed meal and its utilization as a feed ingredient for animal

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Abtract

Rapeseed cake and meal, a by-product of rapeseed, is one of the most important protein feedstuffs. Quality characteristics of rapeseed meal are given as follows: (1) The energy value of rapeseed meal is at variance, for which it is affected by its residual oil, crust and crude fiber content. (2) Rapeseed meal is an excellent protein source owing to its relatively high protein level (30%-40%), lysine, methionine and tryptophan levels as well as its low digestibility. (3) The calcium, phosphorus, selenium, iron and manganese contents in rapeseed meal are very high, but up to 65% of the P content is abound in P-phytate and thus reducing it bioavailability. (4) High levels of nicotinic acid and choline as well as low carotenoid and vitamin D levels are the main vitamins characteristics of rapeseed meal. Furthermore, variety, squeeze technique, anti-nutritional factors and its levels will also influence the nutritional value of rapeseed meal. While the main anti-nutritional factors in rapeseed meal include glucosinolates, sinapine, sinapic acid, tannin, phytic acid and crude fiber, there are some physical, chemical, biological and crop breeding methods to deal with the toxicity in rapeseed meal, of which the crop breeding is the most successful. Chinese "Double low" rapeseed and Canadian canola are main new varieties. Rapeseed meal has been widely used in the diet of some animals, and the amounts incorporated in diets of poultry, swine and cattle are usually 8%-15%, 3%-15% and 5%-20%, respectively. Presently, the anti-nutritional factors in common rapeseed meal, especially the glucosinolates and sinapic acid, are the main limiting factors for its wider application, while popularization and application for new species will bring into an available approach in solving those problems.

Key words: rapeseed meal, rapeseed cake, nutrients, anti-nutritional factors, utilization

Introduction

Rapeseed is one of the most important oil seeds in China, and it is found in abundant in Hubei, Hunan, Sichuan and Jiangsu provinces of China. It is the raw material for rapeseed cake or meal production. Rapeseed meal, the leftover after crushing and squeezing or permeating with solvent, has three kinds of forms namely powder, patch and granular, and is one kind of protein feedstuffs. Although rapeseed meal is one of the most potential protein sources, the inclusion level is difficult to increase in the diet of animals on grounds of the inherent and excessive levels of anti-nutritional factors (glucosinolates, Sinapine and non-starch polysaccharides, etc) in common *Brassica napus* rapeseed meal. The "Double-low" rapeseed (lower glucosinolates and erucic acid similar to Canola) has been introduced in China since the 1990s, and it is promoting the wilder application of the rapeseed meal as a feed ingredient.

The nutritional characteristics of rapeseed meal

The nutrient composition of rapeseed cake and meal is presented in table1.

Table1 The nutrient composition and its content of rapeseed cake and meal

Nutrients	Rapeseed cake	Rapeseed meal
DM (%)	88.0	88.0
CP (%)	35.7	38.6
EE (%)	7.4	1.4
CF (%)	11.4	11.8
NFE (%)	26.3	28.9
ASH (%)	7.2	7.3
NDF (%)	33.3	20.7
ADF (%)	26.0	16.8
Ca (%)	0.59	0.65
TP (%)	0.96	1.02
Non phytate phosphorus (%)	0.33	0.35
Pig digestible energy (MJ/kg)	12.05	10.59
Pig metabolizable energy (MJ/kg)	10.71	9.33
Poultry metabolizable energy (MJ/kg)	8.16	7.41
Beef cattle digestible energy (MJ/kg)	11.51	11.25
Dairy cow net energy (MJ/kg)	5.94	5.82
Sheep digestible energy (MJ/kg)	13.14	12.05

Source: Feng (2003)

Table 2 shows the nutrients composition of the by-products of the common rapeseed, "Double-low" rapeseed meal and rapeseeds. Compared with soybean meal, the crude protein content in rapeseed meal is 3%-8% lower, while the crude fiber content is about 2 times higher. Similarly, the levels of Ca and total phosphorus (TP) are higher too. The ether extract content of the rapeseed meal after expression is lower than that of the raw rapeseed. However, the levels of the other nutrients are comparatively higher as exemplified by crude protein (13.6%-15.0%), crude fiber (3.9%-4.2%), ash content (3.6%-4.2%), etc. The main reason for the higher nutrient content in rapeseed meal is the removal of oil from the rapeseed (38.84%-39.50%). The crude protein content in the "Double-low" rapeseed and its meal is 4.0%-5.4% higher than that in the common rapeseed and its meal. Moreover, the quality of the crude protein in the common rapeseed meal is better than the common rapeseed cake. Which have shown that crude protein contents in the products from rapeseed are affected by the varieties of the rapeseed and treatment methods. The ether extract content is more influenced by the method chosen to squeeze it. For example, the ether extract of rapeseed cake is 4 times higher than that of rapeseed meal from common rapeseed. The ADF and NDF in the "Double-low" rapeseed meal is affected more by the varieties, whose content vary among different varieties, and the coefficients of variance is reportedly by 29.3% and 30.8%, respectively (Peng and Jiang, 1999).

Table 2 The nutrient composition in the rapeseeds, rapeseed cake and meals(%)

Nutrients	Rapeseed meal and cake			Soybean meal (NY/T2)	Rapeseed	
	Common rapeseed meal	Common rapeseed cake	"Double-low" rapeseed meal		Common rapeseed	"Double-low" rapeseed
DM	90.18±0.77	92.34±0.63	91.88±0.55	-	93.00±0.55	94.38±0.11
CP	37.84±0.89	34.78±0.55	40.26±1.13	43.00	20.00±0.05	25.31±0.88
EE	2.04±0.42	8.62±1.06	3.45±0.06	1.90	39.50±0.70	38.84±0.75
NFE	28.30±0.55	28.06±1.51	-	-	-	-
CF	11.92±0.40	10.35±0.65	11.57±0.36	5.10	7.20±0.00	7.38±0.33
NDF	-	-	31.86±5.48	-	-	-
ADF	-	-	23.40±3.62	-	-	-
Ash	8.40±0.76	8.26±0.72	7.90±0.31	6.00	4.20±0.00	4.29±0.27
Ca	0.65±0.01	0.57±0.10	0.66±0.03	0.32	-	0.46±0.00
TP	0.99±0.03	1.12±0.09	1.04±0.02	0.61	-	0.53±0.01

Source: Xi (2002)

Its crust and the crude fiber content, as well as the technique used in expressing the oil is determined the energy content of the rapeseed meal. Also, the level of residual oil influences it. The higher the residual oil, the higher energy level. The energy level of rapeseed meal for pig was reported to be the highest among the values for pig, cattle and poultry, and that for cattle was higher than that for poultry. The digestibility of protein in rapeseed meal is lower than that of soybean meal, but in general, the rapeseed meal is abundant in all kinds of amino acids. Moreover, which is relatively comparable to what prevailed in soybean meal (see table 3). Although the lysine and tryptophan content in rapeseed meal are higher than that of some other kinds of protein ingredients, the arginine content is the lowest. And the methionine content in the feedstuff is higher and only inferior to gingili meal. The ratio between lysine and arginine is about 100:100, which is lower than the ideal ratio (100:120) in animal ration. The rapeseed meal can be also used to balance the amino acid, for example lysine etc. The Ca and P contents are very high, but up to 65% of the phosphorus content is bound to phytate (P-phytate), which makes the phosphorus availability very low. The Se content is 0.16-0.29 mg/kg, and it is 10 times higher than that in soybean meal. The Fe and Mn contents are also high, about 653-687 mg/kg and 78.1-82.2 mg/kg respectively. The niacin (160 mg/kg) and choline (6400-6700 mg/kg) contents in rapeseed has been reported to be very high (Feng, 2003). On the other hand, carotene and vitamin D contents were deficient, whereas thiamin and riboflavin content were found to be relatively low when compared to other plant meals (Feng, 2003).

Table 3 The amino acids composition and content in the rapeseed meal(%)

Component	Rapeseed cake	Rapeseed meal
DM	88.0	88.0
CP	35.7	38.6
Arginine	1.82	1.83
Histidine	0.83	0.86
Isoleucine	1.24	1.29
Leucine	2.26	2.34
Lysine	1.33	1.30
Methionine	0.60	0.63
Cysteine	0.82	0.87
Phenylalanine	1.35	1.45
Tyrosine	0.92	0.97
Threonine	1.40	1.49
Tryptophan	0.42	0.43
Valine	1.62	1.74

Source: Feng (2003)

Although the protein quality of rapeseed meal matches that of the soybean meal, certain essential amino acids, particularly lysine was observed to have a lower bioavailability than that in soybean meal because of the occurrence of Maillard reaction during rapeseed processing into the cake/meal. Zou *et al*. (1992) investigated the rapeseed processing in Sichuan province and found that the highest protein digestibility for pig in relation to three kinds of rapeseed meal were 74.14%, 66.76% and 63.68% for equipment type 95, tape 200 processed and type 95 solvent extract, respectively. It is shown that higher temperature and longer processing time had adverse effects on the protein quality. Li (1993,1995) determined the amino acid content of rapeseed cakes processed by the type 95, 200 and low temperature to be 1.20±0.35%, 1.51±0.25% and 1.76±0.29% respectively, and the mean of the three processing cakes was reportedly 1.24%, yet the rapeseed meal is 1.32%. The mean amino acid bioavailabilities of rapeseed cake, rapeseed meal and low-temperature processing cake were also found to be 84.02%, 86.29% and 90.82% respectively. The lysine bioavailabilities of low-temperature processing cake, rapeseed meal and high-temperature processing cake were 89.1%, 79.2% and 73.3% respectively.

The quality standard of rapeseed cake and meal as animal feed ingredients established in China is mainly based on the crude protein, crude fiber, ether extract and ash levels (see table 4).

Table 4 The quality standard of rapeseed cake and meal as animal feed ingredient in China

Component	No.1		No.2		No.3	
	Rapeseed cake	Rapeseed meal	Rapeseed cake	Rapeseed meal	Rapeseed cake	Rapeseed meal
CP	≥37.0	≥40.0	≥34.0	≥37.0	≥30.0	≥33.0
CF	<14.0	<14.0	<14.0	<14.0	<14.0	<14.0
EE	<10.0	<5.0	<10.0		<10.0	
ASH	<12.0	<6.0	<12.0	<8.0	<12.0	<8.0

Source: GB (10374-89)

The anti-nutritional factors of rapeseed meal and its detoxification

The anti-nutritional factors, which exert adverse effect on growth, health and general welfare of animal, do so through variety of approach to affect digestion, absorbance and availability of the nutrients in the feedstuffs (Huisman and Tolman, 1992). Although the nutrition value of rapeseed meal is approximate to the soybean meal, the application of rapeseed meal is practically limited due to the high levels of some harmful substance as well as many anti-nutritional factors, such as glucosinolates, sinapine and its derivative, tannin, phytic acid, crude fiber etc. Glucosinolates and sinapine are considered to exert the most effect in terms of the application of rapeseed. Glucosinolates, generally exists in the potassium salts form, which include glucosinolates and glucoside, have no anti-nutrition effect of itself. The products of the hydrolysis of glucosinolates with endogenesis thioglucosidase, particularly oxazolidine thione (OZT), thiocyanate, isothiocyanate (ITC) and nitriles are considered to be more harmful than the intact glucosinolates (Wang and Feng, 2000). Even though thioglucosidase in the raw materials becomes in-anctive when processed, the products of rapeseed will be still possible to produce toxicity to animal because the enzyme from some intestinal microorganism has the same active substance similar to thioglucosidase. On the other hand, glucosinolates can be degradated under acid and alkaline conditions too. OZT and ITC are the important anti-nutritional factors because they affect the iodine absorbance, and result in goiter. OZT, referred to as goitrogen, has a strong anti-thyroxine function. While ITC badly influences the palatability of feedstuffs, as well as destroys the exterior of the digestive system. Isothiocyanate strongly affects the rapeseed meal palatability and has a violent stimulatory function to the mucous membrance, which result in gastroenteritis, scours, even pulmonary edema and goiter in pigs because thyroid follicular sets restrained from condensing with iodine. OZT counteracts the synthesizing reaction of thyroxine in non-ruminant animal, which decreases the thyroxine concentration in the blood, and brings about goiter because of thyroid cell hyperplasia in a bid to promote pituitary to excrete more thyrotropin. Thiocyanate affects the conversion of the iodine and results in goiter in the end. Isothiocyanate and thiocyanate are spicy and thus greatly affect the palatability of rapeseed meal. Nitrile is the other metabolites form of glucosinolates. Its toxicity is 10 times greater than that of OZT, and it can make the liver and the kidney intumescent (Slominski *et al*., 1988). When the content in animal ration exceeds 5 mg/g, glucosinolates will adversely affect the animal performance. In a general way, the duck is more sensitive than the broiler, and the broiler is more sensitive than the pig. High glucosinolates content in rapeseed made the broiler liver hemorrhagic (Campbell *et al*., 1991), and pig liver intumescent (Busato *et al*., 1991). Generally, the mean contents of glucosinolates in common rapeseed, *Brassica napus*, *Brassica rapa*, *Brassica juncea* have been reported to be 3%-8%, 6.13%, 4.04%, 4.85% respectively, or expressed as 50-100 μmol/g for all of them (Bell, 1993). The maximum level of glucosinolates in animal ration cannot exceed 2.5 μmol/g, and the level of glucosinolates and its metabolites under 2.4 mmol/kg could not affect pig performance likewise its thyroid function, when 250 μg/kg iodine was added to the pig ration (Schone *et al*., 1991).

Common rapeseed meal contains a lot of glucosinolates, and the main five kinds are 3-bultylene- glucosinolates, 4-amylene-glucosinolates, 2-hydroxide-3-bultylene-glucosinolates, 2-hydroxide-4- amylene-glucosinolates, and 2-allyl-glucosinolates. Those found in *Brassica napus* meal includes 3-bultylene-glucosinolates and 2-hydroxide-3-bultylene-glucosinolates, while *Brassica rapa* includes 3-bultylene-glucosinolates. The levels of glucosinolates in three different kinds of rapeseed are shown in table 5.

Table 5 glucosinolates content and its products content in rapeseed cake and meals

Item	Common rapeseed cake	Common rapeseed meal	"Double low" rapeseed meal
Total glucosinolates(%)	2.42±0.67	0.69	1.35±0.18
OZT (mg/kg)	2566±612	1458	901±271
Isothiocyanate (mg/kg)	2152±694	1423	1165±84
Nitrile (mg/kg)	420±2520	-	

Source: Xi (2002)

Sinapine (4-hydroxide-3,5-dimethyl cinnamic cholinesterase), an ester product formed from the reaction between erucic acid and cholinergic, and another anti-nutritional factor in rapeseed, is instable and can easily be hydrolyzed without the aid of enzyme to get erucic and cholinergic. Sinapine is bitter, and reduces the palatability as well as generate egg's beany flavor because of the aggregation of TMA in eggs. TMA, a sinapine degraded products, is volatile and give off beany flavor. Another character of sinapine is that it is easily hydrolyzed. Currently, erucic acid has little effect on the performances of animal except that when animals take a lot of it, it induces fat aggregation in cardiac muscle and cardiac muscle putrescence. Sinapine content is only about 1% in common rapeseed meal and is 0.6%-1.8% in "Double low" rapeseed meal (Bell, 1993).

Erucic acid, no sinapine (the product of sinapine hydrolysis), is another anti-nutritional factor that found in crucifer crop, such as rapeseed. The erucic acid content in Chinese common varieties is very high. Erucic acid is composed by 22-carbon atom and the unsaturated fatty acid with 1 double bond. The erucic acid contents are 22%-66% in rape oil from common rapeseed and reduce to 2.6% in "Double low" rapeseed meal (Chen, 2003). As a toxin, erucic acid can slow growth, cause hypogenesis, tumescence of heart, change fat content of the heart, impair cardiac muscle, increase death rate, decrease thrombocyte content as well as reproductive ability. Using male rats, it was shown that these were decrease in spermatogenesis and immaturity of sperms when the erucic acid content in the rat ration was more than 10%.

The mean content of tannin in common rapeseed cake, common rapeseed meal and "Double low" rapeseed meal have been variously but separately reported to be about 0.62% (Wang, 1988; Li *et al*., 1993; Shao *et al*., 1992), 0.52%(Li *et al*., 1993) and 0.65% (Peng, 1999; Qing, 2005) respectively. Tannin tastes saline and affects its palatability. However, tannin in rapeseed meal has little effect on palatability compared to that in sorghum. Moreover, tannin can combine with digestive enzyme, especially pepsin, and decrease the availability of protein in rapeseed meal.

The phosphorous content in rapeseed meal is higher than some other plant feed ingredients, and the total phosphorous content can reach up to about 1%, but the most are phytate phosphorus. The highest contents of phytic acid and phytate phosphorus in common rapeseed meal is up to 2% and 0.67% respectively (Feed Databank in China, 2005). "Double low" rapeseed meal has good effect on decreasing glucosinolates and erucic acid content, and increase phytic acid content as well. In general, total phosphorous content in "Double low" rapeseed meal is 0.94%-1.13%, while phytate phosphorus content reaches 72%-77% of the total phosphorous. Phytic acid cannot be digested in the gut of non-ruminants, which tends to reduce the availability of phosphorus. Thus phytate reduce the digestibility of protein and essential mineral elements because of complexing them, especially Zn which is reduced to 44.1%.

The availability of energy in rapeseed is low, due to its high fibre content. Compared with conventional varieties, "Double low" varieties, with low content of erucic acid and glucosinolates, have a disadvantage owing to the presence of fibre, especially NSP that is considered as an anti-nutrient. The level of NSP, cellulose and other NSP, are 17.9%, 4.9% and 13.8% respectively. Other NSP are usually made up of arabinoxylans, xylose, galactose, uronic acid and mannose, etc (Slominski and Campbell, 1990). Other compositions in fibre are some oligosaccharide. Bell and Hicklin (1999) reported that the contents of NSP, CF, ADF and NDF in rapeseed were 16.1%, 12.0%, 17.2% and 21.2%, respectively. While the levels of CF, NDF, ADF were 1.4%, 33.3% and 26.0% in rapeseed cake, and 11.8%, 20.7% and 16.8% in rapeseed meal respectively (Feed Databank in China, 2005). Peng (2000) indicated that the ADF, NSF, NDSP, AND in four "double low" varieties were 18.6%-22.6%, 24.4%-30.1% and 7.0%-9.1%, respectively. These results show that high content of fibre affects the energy value in rapeseed.

The alexipharmic methods of rapeseed meal are physical, chemical, biological method and crop breeding (Wang and Feng, 2000). Physical methods include inactivation of myrosinase and shelling, and the former consisted of steam heating, roasting, microwave treatment and bulking etc. For the methods cannot stop reaction of enzymes hydrolyzing erucic in animal, physical methods are rarely applied in real production. Milling with water can activate myrosinase to reduce the glucosinolates. Drying enhances volatilization of isothiocyanates, while extraction causes glucosinolates to survive by 40%-80% in rapeseed meal (Schone *et al*., 1996). Heat treatment inactivates myrosinase, and Schone *et al*. (1993) considered that supplementation of enzymes from microorganism was indispensable to hydrolyze glucosinolates. Other investigators held contrary viewpoint that inactivating myrosinase caused glucosinolates to be stabilized which improved the rate of oil yield (Liu *et al*., 1994). Jensen *et al*. (1995) reported that heat treatment at 100°C for 15, 30, 60 and 120 min reduced the level of glucosinolates by 24%, 46%, 70%, 95% respectively, and the solubility of protein as well. In general, heat treatment at 100°C for 30 min was the best.

The simplest method of detoxifying rapeseed meal is by immersion in water. The treatment with water (1:6) for 15-20 min can decrease glucosinolates by 98% and DM by 30% (Fauduct *et al*., 1995). Thacker (1998) reported that microwave treatment of intact rapeseed improved its nutrient values in the diet for pigs, which was attributed to the reduction in the activities of myrosinase. Shelling can reduce the toxin content, and invariably decrease protein content.

Acid and alkaline degradation, metal salts degradation and solvent extractions constitute the chemical method. Acid and

alkaline degradation reduced toxicity when H_2SO_4, NaOH, KOH, NH_3, $Ca(OH)_2$ were used, however these treatments require heat treatment, expensive and goes with pollution. The rapeseed meals from acid and alkaline degradation are of poor quality and also affect the palatability of animal. Metal salts degradation catalyze glucosinolates hydrolyzation with metal salts such as iron, copper and so on. The second method only works in reducing glucosinolates content. In the process of solvent extraction, the solvents including ethanol, carbinol, acetone and water etc., decreases lower molecular weight glucosinates.

Biological methods usually include fermentation and hydrolyzation catalyzed by enzyme. In the case of the former, rapeseed meal is fermented with yeast or other microorganism to reduce the toxin. The latter catalyzes the hydrolysis of glucosinolates with activators (such as vitamin C etc.) and enzymes in rapeseed meal.

Crop breeding is one way to develop new varieties with low erucic acid and glucosinolates, which is the most successful among the four methods. "Double low" rapeseed is an excellent protein feedstuff for livestock owing to its high protein content and well-balanced amino acids. In a study by Bell (1993) on Canola, it was shown that the glucosinolates content was below 30 μmol/g based on dry matter-ont-fat, and the erucic acid content was below 2% based on total fatty acid (50-100 μmol/g and 25%-45% in common varieties). However, the biosafety of gene-improved new varieties should be given more attention.

Utilization of rapeseed meal in animal ration

If undetoxified rapeseed meal containing the anti-nutritional agents is used in chicken rations, then severe struma, bleeding of liver may happen. This is shown in most of chicken breeds with the white leghorn showing the most severe response. The common rapeseed meal is not usually used in formulating diets for young chickens. 10%-15% of high-quality rapeseed meals are aptly used in later stage diet for broilers. In order to avoid debasing the quality of chicken, less than 10% of rapeseed meal in diet for broilers should be chosen. As for laying hens, 8% of rapeseed meal in the diet is suitable, and 12% level will reduce egg weight as well as the rate of hatch. Superfluous rapeseed meal results in similar bad smell similar to rotten fish, the reason of which is that microorganism in the gut of chicken will turn sinapine into trimethylamine with the consequent bad smell. Rapeseed meals with high content of glucosinolates lead to iodine deficiency disorders of young chicken on day 9. The energy value of it is much various with animal, mensuration method and so on, which is different with other plant-protein feedstuffs. In different growing phase of broilers, ME values of rapeseed are different, for example 1375 Kcal/kg for young chicken vs. 2265Kcal/kg for adult broiler (Japanese feeding standards, 1975). ME of rapeseed meal for chicken is reported to be 1630 Kcal/kg according to Japanese feeding standards (1975), and 2040 Kcal/kg according to NRC (1984). ME of rapeseed cake and rapeseed meal in Chinese feeding standards is usually 8.16 MJ/kg and 7.41 MJ/kg, respectively.

In recent years, "Double low" varieties have gained much attention by way of the quantities that are planted in China. However, in many cases the presence of glucosinolates in rapeseed meal is still considered as a factor re-stricting its inclusion level in the diet for non-ruminants, owing to both the physiological and anti-nutritional impact. For example, Slominski and Campbell (1991) reported that goiter and liver tumescence were observed in chicken and rat respectively when 30% of rapeseed meal was added to their rations.

Theoretically, complex enzymes including xylanase and α-galactanase can improve nutrient utilization because of the presence of high level of α-galactan (2%-9%) and NSP, especially xylan in rapeseed meal. Some soybean oligosaccharides, raffinose and stachyose about 2.5% are observed in this feedstuff (Slominski and Campbell, 1991). Slominski *et al*. (1994) reported that the digestibility of NSP in Canola meal with low oligosaccharides was higher than that of common Canola varieties. Bedford and Morgam (1995) indicated that xylanase supplemented in diet with canola meal improved the performance of broilers. Feng and Shen (2005) set up the system of "effective nutrients improvement value (ENIV)" to calculate nutrient values of feedstuff supplemented with enzymes. The ENIV values of ME and protein for poultry fed rapeseed meal supplemented with xylanase and cellulase etc are about 120-170 Kcal/kg and 3.5%-5.2%, respectively.

DE values of rapeseed cake and meal for swine are 12.5 MJ/kg and 10.59 MJ/kg respectively. The characteristic of common varieties of rapeseed meal are then not being palatable to swine, which bring thyroid, kidney and liver tumefaction, reduction of growth rate by 30%, decline of reproduction performance of sow. Fitting amount of common rapeseed meal used in diet are 5% for hog, 3% for sow, and that of "Double low" varieties are 15% for hog, 12% for boar, more than 10% for hog without fat softening. Supplemented with premix enzymes containing xylanase and cellulase, the ENIV values of ME and digestible protein in rapeseed meal were 100-135 Kcal/kg and 3.5%-5.2 % (Feng and Shen, 2005).

Though rapeseed meal used in cattle diet reduces palatability and results in goiter, its effect is not much in contrast with non-ruminants. The amounts of this feedstuff used in the diets are 5%-20% for beef cattle and less than 10% for cow with normal milk yield and milk fat. New varieties of rapeseed meal with better feeding effect, can be increased the amount in cattle ration.

References

Bedford M.R. and Morgan A.J. 1995. The use of enzymes in canola-based diets. In: van Hartingsveldt, W., Hessing,M., van der Lugt, J.P. and Somers, W.A.C. (eds) 2nd European Symposium on Feed Enzymes. Proceedings of ESFE2, Noord Wijkerhout, The Netherlands, pp.125-131.

Bell J.M. 1993.Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73:679-697.

Busato A.; Bestetti G.E. and Rossi G.L. 1991. Effects of feeding rapeseed meal on liver and thyroid gland function and histomorphology in growing pigs.J. Anim. Physiol. Anim. Nutri. 66:12-27.

Campbell L.B. and Slominski B.A.1991. Nutritive quality of low-glucosinolates canola meal for laying hens "Proc. 8th Int. Rapeseed Congress".pp 442-447.Saskatoon,Canada.

Chen Gang. 2003. Evaluation of quality characters, quality Influencing factors and processing technics of chinese rapeseed cake and meal. The master's degree

dissertation of Huazhong Agricultural University (in Chinese).
Chinese Feed Databases. 2005. The table of Chinese feed ingredient and its nutritional values (16th edition). China Feed, 21:21-31 (in Chinese).
FAO / WHO. 1973. Energy and Protein Requirement. WHO Tech. Report series[R]. N0.552, Geneva.
Fauduct H.; Coic J.P. and Lessire M.1995. Rapeseed meal upgrading Pilot Scale Preparation of rapeseed meal materials with high or low glucosinolate contents. Anim. Feed Sci. Technol. 56:99-109.
Feng Dingyuan and Shen Shuibao. 2005. New theoretic of feed enzyme: the establishment and application of ENIV system. Application of Enzymes in Feed Industry (Feng Dingyuan edited). China Agricultural Scientific and Technology Press. pp7-8 (in Chinese).
Feng Dingyuan. 2003. Compound Feed Science. China Agricultural Press, pp78-80 (in Chinese).
Huisman J. and Tolman G.H.1992. Antinutritional factors in the plant proteins of diets for non-ruminants. In: Recent Advances in Animal Nutrition 1992.
Jensen S.K.; Liu Y.G. and Eggum B.O.1995. The effect of heat treatment on glucosinolates and nutritional value of rapeseed meal in rats. Anim. Feed Sci.Technol. 53:17-28.
Li Jianfan, Gao Zhenchuan and Jiang Yunxia, et al. 1995. The nutritions and anti-nutritional factors of rapeseed meals in china. Acta Veterinariaet Zootechnica Sinica, 26(3): 193-199 (in Chinese).
Li Jianfan, Gao Zhenchuan and Jiang Yunxia. 1993. Effect of different processing methods on the contents and availability of amino acids in rapeseed meal. Scientia Agricultura Sinica, 26: 68-79 (in Chinese).
Liu Y.G.; Steg. A. and Smits B. et al. 1994. Crambe meal: removal of glucosinolates by heating with additives and water extraction. Anim. Feed Sci. Technol. 48:273-287.
Peng Jian and Jiang Siwen. 1999. Effects of dietary fibre on digestibility and availability of amino acids in pig. Pigs and Poultry, 5:10-12 (in Chinese).
Peng Jian. 2000. Evaluation and improvement of quality of Chinese double-low rapeseed meal. The Ph.D dissertation of Huazhong Agricultural University (in Chinese).
Qing Zhongquan. 2005. Study on nutritional characters and application of double low rapeseed meal in pig. The master's degree dissertation of Huazhong Agricultural University (in Chinese).
Schone F.; Jahreis G. and Richter G. et al 1993. Evaluation of rapeseed meals in broiler chicks-effect of iodine supply and glucosinolate degradation by myrosinase or copper. J. Sci. Food Agric. 60:245-252.
Schone F.; Ludke H.and Geinitz D.et al. 1991. Effect of low or high glucosinolate rapeseed meal on growth, thyroid hormone, vitamin A and trace element status of pigs. Proc. 8th International Rapeseed Congress, Saskatoon, Canada. pp1577-1584.
Schone F; Kirchheim V. and Schumann W.1996. Apparent digestibility of high fat rapeseed press cake in growing pigs and effects on feed intake, growth and weight of thyroid and liver. Anim. Feed Sci. Technol. 62:97-110.
Shao Chunrong and Bao Chengyu. 1992. The distributing rule and affect factors of Anti-nutritional factors for rapeseed cake in Jiangsu province. Proceedings of Animal Nutrition. China Agricultural Press, in Beijing. pp730 (in Chinese).
Slominski B.A. and Campbell L.D. 1991. The carbohydrate content of yellow-seeded canola. In: Proc. 8th Int. Rapeseed Congress. pp1402-1407.
Slominski B.A.; Campbell L.D. and Stanger N.E.1988. Extent of hydrolysis in the intestinal tract and potential absorption of intact giucosinolates in laying hens. J. Sci.Food Agric. 42:305-314
Slominski, B.A. *et al*. 1994. Oligosaccharides in canola meal and their effect on nonstarch polysaccharide digestibility and true metabolizable energy in poultry. Poultry Sci., 73:156-162.
Thacker P.A.1998. Effect of micronization of full-fat canola seed on performance and carcass characteristics of growing-finishing pigs. Anim. Feed Sci. Technol. 71:89-97
The Bureau of Animal and Veterinary of Ministry of Agriculture of the People's Republic of China, et al. 2002. The standard compilation of feed industry. Standards Press of China. Pp446-449 (in Chinese).
Wang Daoshun. 1988. Exploiture and application of cotton and eapeseed meal. Hubei Agricultural Sciences, 11: 18-20 (in Chinese).
Wang Jianhua and Feng Dingyuan. 2000. Feed Hygiene. Xi'an map press. pp98-120 (in Chinese).
Xi Pengbin, Li Defa and Gong Limin. 2002. The nutritional value of rapeseed meal and its application in diet for swine. Feed Industry, 32(6): 5-9 (in Chinese).
Xi Pengbin. 2002. Composition characteristics and ileal amino acid digestibility of Chinese rapeseed meals in growing pigs. The Ph.D dissertation of China Agricultural University (in Chinese).

Influence of full fat rape seed on the fatty acid profile of egg yolk fat (Q1)

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Abstract

A feeding trial with brown laying hens (Lohmann Brown) was carried out at inclusion levels of 0 % (I), 7.5 % (II), 15 % (III), 22.5 % (IV) and 30 % (V) chemical and hydrothermical treated full fat rape seed in the diets. The diets were based on wheat and soybean meal and fed to 72 hens per group during the period from 27th to 62nd week of age.

The fatty acid profile of egg yolk was markedly influenced by rape seed oil. Despite eggs of group I (without rape seed) the content of saturated fatty acids in the yolk fat decreased and the content of n-6 and n-3 fatty acids increased by increasing inclusion levels of full fat rape seed in the diets. Because the n-3 fatty acids level increased more rapidly than n-6 acids, the ratio n-6 to n-3 fatty acids in the egg was narrowed.

Key words: laying hens, full fat rape seed, egg yolk, fatty acids

Introduction

The human consumption of n-3 fatty acids (also called omega-3 fatty acids) is lower than the recommendations, still insufficient and it can cause the occurrence of heart-related diseases (Jahreis, 2003). It is known that the fatty acid profile of eggs can be modified by feeding of hens (Ternes et al., 1994). In the last decade trials were carried out in order to enrich the hen eggs with those essential fatty acids, especially n-3 fatty acids (Brettschneider, 2006). However, there was indication of sensoric deviations in hens eggs by using of fish or flaxseed oil in hen diets (Scheideler et al., 1997, Van Elswyk et al., 1995). The rape seed oil is also rich in alpha-linolenic acid with very low ratio of linoleic n-6 fatty acid to α-linolenic n-3 fatty acid 1.7 to 1 (Jahreis, 2003). Therefore, in experiments with full fat rape seed as compound in laying hen diets should also be confirmed the thesis of the influence of fatty acid profile in egg by intake of oil in full fat rape seed.

Materials and Methods

Chemical and hydrothermical treated rape seed from "LIRAJET" double zero strain was tested in a feeding trial with Lohmann Brown laying hens. Rape seed was included in the levels of 0 % (group I), 7.5 % (group II), 15 % (group III), 22.5 % (group IV) and 30 % (group V). The experimental diets contained wheat, soybean meal, wheat bran (Table 1). The rape seed contained < 50 mg sinapine and 1,4 mmol glucosinolate/ kg (91 % dm).

Table 1. Composition of the experimental diets

Components (g/kg)	Group				
	I	II	III	IV	V
Wheat	779	628	487	304	136
Wheat bran	-	79	162	304	418
Soybean meal		84	97	38	44
HP - soybean meal	124	38	10	36	11
Rape seed	-	75	150	225	300
Calcium carbonate	69	72	74	76	77
Calcium phosphate	14	11	8	6	3
L-Lysine-HCl	2	1	1	-	-
DL-Methionine	1	1	-	-	-
Sodium chloride	1	1	1	1	1
Premix [1]	10	10	10	10	10
Composition (*calculated, ** - analysed)					
AME_N (MJ)*	11.1	11.2	11.3	11.1	11.1
Crude protein**	177.0	180.0	178.5	183.0	182.0
Crude fat**	21.0	57.0	79.5	126.5	156.0

[1] Supplements per kg of diet: 25 mg Fe, 60 mg Mn, 75 mg Zn, 5 mg Cu, 0.5 mg I, 0.1 mg Se, 0.1 mg Co, 10000 IE vit. A, 2000 IE vit. D_3, 10 mg vit. E, 2.5mg vit. K, 1 mg vit. B_1, 4 mg vit. B_2, 3 mg vit. B_6, 10 µg vit. B_{12}, 25 mg niacin, 10 mg pantothenic acid, 400 mg cholin chloride, 5 mg canthaxanthine

Each group was assigned into 8 subgroups of 9 hens each (72 hens per group). All hens were kept in single cages. The

subgroups were equal randomised into the 3-floor-batteries. The trial was carried out over 36 weeks (from 27th to 62nd week of age). In intervals of 4 weeks (9 times) 36 eggs from each group were collected for fatty acid profile analysis. After estimation of egg weight the egg yolks were homogenised and frozen dried for analysis. Fat extracts from feeds and eggs were methylated with trimethylsulfoniumhydroxide and the resulting methyl esters were identified from their retention time using a gas-chromatography system (HP 5890 gas-chromatograph, HP 7673 auto sampler, HP 3365 data-station and flame ionisation detector). Analysis of variance and comparisons of mean differences between groups (Tukey HSD-Test) were performed using Statistica for Windows Operating System (Statsoft Inc., 1996).

Results and Discussion

Intake of feed and fatty acids. The daily feed intake was not influenced by rape seed inclusion level (Table 2). By graded inclusion levels of rape seed in the diets the intake of oleic acid, linoleic acid and α-linolenic acid was increased

Table 2. Feed intake and consumption of oleic acid (C 18:1), linoleic acid (C 18:2) and α-linolenic acid (C 18:3), average value for 27th – 62nd week

Group	Feed intake	Consumption (mg/hen/day) [2]		
	g/hen/day	C 18:1	C 18:2	C 18:3
I (0[1])	117 ± 9	257	936	54
II (7.5)	115 ± 8	2507	1806	391
III (15.0)	115 ± 7	4680	2438	805
IV (22.5)	119 ± 7	6771	3368	1154
V (30.0)	118 ± 6	8779	3918	1499

[1] Rape seed in % of the feed mixtures
[2] Calculations on the basis of feed intake and fatty acid contents of the feed mixtures

Fatty acids profile of the egg yolk. In table 3 the results from the estimation of the fatty acid profile of egg yolk fat was shown.

Table 3. Fatty acid composition of the egg fat

Fatty acid (%)	Group				
	I (0[1])	II (7.5)	III (15.0)	IV (22.5)	V (30.0)
Myristic	0.4 [b]	0.3 [b]	0.3 [b]	0.2 [a]	0.2 [a]
Palmitic	21.6 [d]	20.4 [cd]	18.8 [bc]	17.8 [ab]	16.8 [a]
Palmitoleic	4.2 [c]	3.0 [bc]	2.4 [ab]	2.2 [ab]	1.3 [a]
Stearic	5.6 [c]	5.3 [bc]	4.8 [ab]	4.5 [a]	4.3 [a]
Oleic	44.5	44.4	44.6	45.1	45.3
Linoleic	9.3 [a]	11.0 [ab]	12.7 [bc]	13.6 [c]	14.6 [c]
α-Linolenic	0.8 [a]	1.3 [a]	1.8 [ab]	2.1 [bc]	2.3 [c]
Arachidonic	0.28	0.22	0.28	0.34	0.32
Eicosapentaenoic	0.05	0.05	0.06	0.06	0.07
Docosahexaenoic	0.35 [a]	0.48 [b]	0.56 [bc]	0.55 [bc]	0.61 [c]

[1] Rape seed in % of the feed mixtures
[a-d] different letters denote significant differences in fatty acid ($p<0.05$)

The fatty acids profile of the control group correlated with references of Ternes et al. (1994). By feeding diets less in fat and rich in carbohydrates (e.g. group I) the self synthesis of fatty acids dominated. In this case saturated chains of fatty acids were deposed in eggs (mainly palmitic C 16:0 and oleic C 18:1). By higher inclusion levels of rape seed into diets the fatty acids profile of egg yolk changed markedly. The level of saturated fatty acids in egg fat decreased and increased both linoleic and α-linolenic fatty acids but the content of oleic acid was not influenced. The content of alpha-linolenic fatty acid increased more than linoleic acid and leaded to decrease of the ratio n-6 to n-3 fatty acids in egg fat at higher inclusion levels of rape seed in the diets. The increased finding of eicosapentaenoic acid (C 20:5 n-3) and docosahexaenoic acid (C 22:6 n-3) was caused by enzyme systems in hen's metabolism. Such polyunsaturated fatty acids were formed (Nollet, 2001) by desaturation and elongation from alpha-linolenic (precursor n-3) and linoleic fatty acids (precursor n-6). This synthesis was not very efficient as shown the content of polyunsaturated fatty acids (Table 3). The content of n-6 and n-3 fatty acids in egg (60 g) was calculated (in groups I – V respectively): 564, 675, 784, 839 and 898 mg n-6 fatty acids and 75, 112, 148, 165 and 182 mg n-3 fatty acids.

Transfer rate of linoleic and alpha-linolenic acid in the egg. The kind of criteria for valuation of the efficiency of modification fatty acid profile in the egg yolk by rape seed oil could be the transfer rate of the essential fatty acids from feed to egg yolk (Table 4). High transfer (%) of polyunsaturated (C 18:2 and C 18:3) fatty acids was found in group I and the transfer (%) decreased markedly at higher intake of linoleic and alpha-linolenic acids. Eder et al. (1998) estimated a transfer rate for alpha-linolenic acid between 15 and 20 % when the hen's diet contains 10 % flaxseed or 10 % flaxseed oil.

Table 4. Transfer of linoleic (C 18:2, n-6) and α-linolenic (C 18:3, n-3) acids from the feed into the egg

Items	Group				
	I (0[1])	II (7.5)	III (15.0)	IV (22.5)	V (30.0)
Intake (mg/hen/day):					
Linoleic acid	936	1806	2438	3368	3918
α-Linolenic acid	54	391	805	1154	1499
Daily egg mass production (g/hen)	54.6	54.2	53.3	52.2	49.2
Content in daily egg mass (mg):					
Linoleic acid	491	588	670	699	703
α-Linolenic acid	42	70	95	108	111
Transfer (%):					
Linoleic acid	52	33	27	20	18
α-Linolenic acid	78	18	12	9	7

[1] Rape seed in % of the feed mixtures

Conclusions

An improvement of dietetic value of eggs by including 15% of rape seed (practice recommendation of treated rape seed for brown layers) in feed was shown and egg contains 148 mg n-3 fatty acids. The continuous consumption of such eggs can improve the ratio of n-6 to n-3 fatty acids in the human nutrition.

Acknowledgements

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References

Brettschneider, J. G., 2006: Influence of chemical-hydrothermal treated rapeseed on performance, egg quality and parameters of thyroid gland of layers. Ph. D thesis University Warmia and Mazury, Olsztyn, Poland.

Eder, K., D. A. Roth-Maier and M. Kirchgeßner, 1997: Laying performance and fatty acid composition of egg yolk lipids of hens fed diets with various amounts of ground or whole flaxseed. Arch. Geflügelk. 62, 223-228.

Jahreis, G., 2003. Physiological effects of plant oils in human nutrition. UFOP-Schriften, Öl- und Faserpflanzen (Oil 2000). 20, 91-99.

Nollet, L., 2001. Modification of the yolk fatty acid profile for the health conscious consumer. In: Proc. 13th Eur. Symp. Poult. Nutr., Blankenberghe, Belgium, 53-60.

Scheideler, S. E., G. Froning and S. Cuppert, 1997: Studies of consumer acceptance of high omega-3 fatty acid-enriched eggs. J. Appl. Poultry Res. 6, 137-146.

Statsoft Inc., 1996. Statistics for the Windows™ Operating System, Tulsa OK, USA.

Ternes, W., L. Acker and S. Scholtyssek, 1994: Ei und Eiprodukte. Parey Verlag, Berlin - Hamburg, pp. 34-35.

Van Elswyk, M.E., P.L. Dawson and A. R. Sams, 1995: Dietary menhaden oil influences sensory characteristics and headspace volatiles of shell eggs. J. Food Sci. 60, 85-89.

Canola protein concentrate for use as a high-valued animal feed ingredient

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Abstract

Conventional processing of canola (*Brassica napus*) generates a high valued oil plus a low valued protein containing meal. The meal is generally used as a feed ingredient for livestock and the low value is a direct result of high levels of fiber and antinutritional factors such as phytic acid. One method of increasing the value of canola is to develop a process to concentrate the protein into high valued products low in fiber and antinutritional factors. MCN BioProducts Inc. has developed a proprietary process for the production of canola protein concentrates. An insoluble canola protein concentrate (IP) and a soluble canola protein concentrate (SP) are produced from solvent extracted starting material. The process can also be applied to non-solvent expelled canola cake with the production of the IP product. The products are greater than 60% protein, have 0 detectable phytic acid and less than 5 μmole/g of total glucosinolates. The protein concentrate obtained from non-solvent expelled canola meal contains 5-10% crude fat. The balance of essential amino acids on a % of nitrogen basis in the protein concentrate products resembled that of the starting material. The products have utility in diets for carnivorous fish and other animal species requiring high valued, highly digestible protein concentrates of plant origin.

Key words: canola protein concentrate, rapeseed protein concentrate, phytate

Introduction

MCN BioProducts has developed a proprietary process for the fractionation and production of high valued protein concentrates from solvent extracted canola white flake and from non-solvent expelled meal. The paper describes dry matter, protein and crude fat mass flows and the nutrient and antinutrient contents of the protein concentrate and by-products outputs.

Materials and Methods

Solvent laden oil extracted canola was obtained from a local crushing facility. This material was air-desolventized to generate non-toasted solvent extracted canola white flake. Whole seed canola was obtained from a local supplier and processed through a non-solvent double press procedure (POS Pilot Plant, Saskatoon, SK, Canada) consisting of flaking, conditioning, pre-press the full press expelling. The white flake or non-solvent expelled cake was initially slurried in water and then processed through the proprietary MCN aqueous fraction process to generate the protein concentrate and by-product streams. Dry matter and crude fat mass flows were monitored through the process. Dry matter was determined by weight differential upon evaporation of moisture using an HB43 Halogen Automated Moisture Analyzer. Crude fat was determined by solvent extraction using an Ankon XT20 automated fat analyzer. Glucosinolates and phytates were determined by high pressure liquid chromatography.

Results

1. Solvent Extracted White Flake Process.

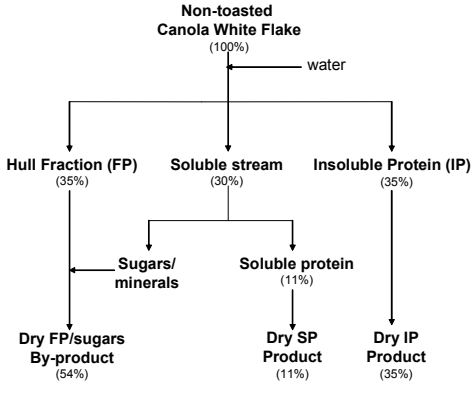

Fig. 1. Aqueous fractionation of non-toasted canola white flake.

1.1 Mass Flows. Figure 1 outlines the product streams obtained from the aqueous fractionation scheme applied to non-toasted solvent extracted canola white flake. The process generates a hull enriched by-product fraction (FP), a fraction enriched in insoluble protein (IP) and a third fraction containing residual solubles. The solubles stream is then further fractionated to generate a concentrate of soluble protein (SP) and a by-product stream enriched in sugars and minerals. The by-products streams can be mixed and co-dried or dried separately.

Table 1 summarizes the mass flows obtained from 4 separate runs of the process. The combined by-product streams account for an average of 51% of dry matter and 21% of protein mass flows. The dry matter protein content averaged 70% for the IP product and 66% for the SP product.

Table 1. Dry matter and protein flows through the aqueous fractionation process*.

	Protein (% of d.m.)	Mass flows (% of White Flake)	
	Protein	Dry matter	Protein
Starting White Flake	42.0		
FP/sugars	17.4±1.0	51.3±6.7	21.3±1.4
Insoluble Protein	69.6±3.1	33.5±4.6	55.5±7.3
Soluble Protein	66.0±4.2	9.8±2.1	15.4±1.0
Total % recovery		94.6	92.2

*Results are expressed as the mean and standard deviation obtained from triplicate runs of the proprietary fractionation process.

2.1 Product Compositions and protein solubility. Table 2 shows the key nutrient and antinutrient contents of the 3 product streams. The process includes a dephytinization step applied after removal of the hull fraction. This step results in 0 detectable phytic acid in the protein concentrates and the sugars/mineral by-product stream. The phosphate associated with the phytate in the starting material is largely converted to available inorganic phosphate. Glucosinolates and other water soluble antinutritional factors are washed from both the insoluble and soluble protein concentrates during the process. The balance of essential amino acids expressed on a per unit of protein basis is similar to that of canola meal. The protein dispersibility index (PDI) values were consistent with the water solubility of the products.

Table 2. Composition of Product Streams from Aqueous Fractionation of Canola White Flake (typical single batch analysis)

Composition (% of d.m.)	Insoluble Protein Concentrate (IP)	Soluble Protein Concentrate (SP)	FP/sugars By-product
Protein	69.7	63.1	21.9
PDI*	4.8	72.3	----
Crude Fiber	3.98	0.45	16.9
Ether Extract	0.27	0.23	0.40
Ash	10.4	10.2	10.2
Antinutritionals			
Phytic acid	0.00	0.00	0.60
Glucosinolates (umole/g)	3.53	4.01	3.58

*Protein Dispersibility Index (soluble protein as a percentage of total protein in the sample)

2 Non-Solvent Expelled Meal Process

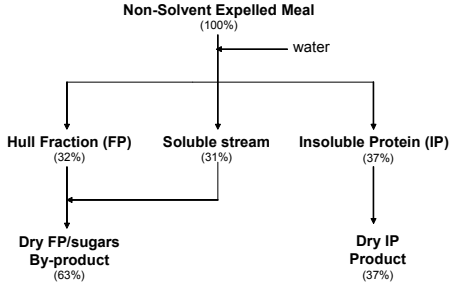

Fig. 2. Aqueous fractionation of non-solvent extracted expelled canola meal.

2.1 Mass Flows. Figure 2 outlines the product streams obtained from the aqueous fractionation of non-solvent expelled canola meal. This process differs from that applied to canola white flake in that a concentrate of soluble protein is not produced. The soluble stream can be mixed with the hull enriched by-product fraction as shown in figure 2 or can be dried separately. The concentrate of insoluble protein (IP) is the high valued product output from the core process.

Table 3 summarizes the mass flows obtained from 3 separate runs of the process. The starting material contained 40% protein and 12% crude fat. The high valued protein concentrate fraction accounted for 36% of dry matter, 56% of protein and 33% of crude fat flows from the expelled cake starting material. The solubles stream accounted for 40% of the original crude

fat in the expelled meal.

Table 3. Dry matter, protein and crude fat flows through the aqueous fractionation process*

	Composition (% of d.m.)		Mass flows (% of starting cake)		
	Protein	Crude fat	d.m.	Protein	Crude fat
Starting cake	39.8	12.4			
Hull fraction	31.8±1.0	6.25±0.9	26.3±1.7	21.3±1.4	14.5±1.0
Protein concentrate	61.9±1.1	10.0±0.8	36.2±4.6	55.5±7.3	33.2±6.6
Solubles stream	13.1±0.4	12.0±1.0	36.9±3.1	11.9±1.0	40.4±6.4
Total % recovery			99.4	88.7	88.1

*Results are expressed as the mean and standard deviation obtained from triplicate runs of the proprietary fractionation process.

2.2 Product Composition. Table 4 shows the key nutrient and antinutrient contents of the 3 product streams from the process. Phytate is removed from the dehulled extract and soluble antinutritional factors are washed from the high valued protein concentrate. The residual crude fat in the expelled cake starting material accumulates in the solubles stream and, to a lesser extend, in the protein concentrate outputs from the process.

Table 4. Composition of Product Streams from Aqueous Fractionation of Non-Solvent Expelled Canola Meal (typical single batch analysis)

Composition (% of d.m.)	Insoluble Protein Concentrate (IP)	Hull Fraction (FP)	Solubles
Protein	67.4	32.5	9.7
PDI*	3.2	---	----
Crude Fiber	6.61	26.9	0.20
Ether Extract	5.65	7.42	11.6
Ash	5.78	3.06	34.8
Antinutritionals			
Phytic acid	0.00	0.00	0.0
Glucosinolates (umole/g)	3.74	1.09	11.3

*Protein Dispersibility Index (soluble protein as a percentage of total protein in the sample)

Discussion

The protein concentrate products generated by the processes are initially targeted for use as high value feed ingredients in diets for carnivorous farmed fish diets and in other animal feeding applications requiring highly digestibly, high quality protein concentrates. Previous work demonstrated that dephytinized canola protein concentrates can replace up to 50% of the fishmeal diets fed to rainbow trout with no effects on any measured parameter (Thiessen et. al. 2004). An earlier study found that, with inclusion of attractant, dephytinized canola protein concentrates can replace 100% of fishmeal in diets for rainbow trout without compromising performance (Higgs et al 1995). These studies indicate the feeding value of canola protein concentrates.

References

Theissen, D.L, D.D. Maenz, R.W. Newkirk, H.L. Classen and M.D. Drew, 2004. Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout (*Onchohynchus mykiss)*. Aqua. Nutr. 10:379-388.

Higgs, D.A., B.S. Dosanjh, A.F. Prendergast, R.M. Beams, R.W. Hardy, R.W. Riley and G. Deacon. 1995. Use of rapeseed/canola protein products in finfish diets. In: Nutrition and Utilization Technology in Aquaculture (Lim, C.E. and Sessa, D.J. eds) pp. 130-156. AOCS Press Champaign Il.

Heat treatment of rapeseed as an alternative to formaldehyde use for protecting proteins in rumen

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Abstract

Formaldehyde treatment is currently used as a procedure to protect the proteins of feedstuffs in rumen and decrease, in this way, their degradability. 60% of rapeseed meal actually used in France by dairy cows are treated by formaldehyde. Nevertheless, questions about risks of formaldehyde for consumers have been recently raised. If this chemical compound ought to be forbidden by European regulations, alternative treatments of meals must be used and prove a technical and economical equivalence with the actual practice. The objective of this study was to evaluate alternative ways to the traditional crushing process : 1) for protecting rapeseed proteins through heating treatments, 2) with preserving a good quality for crude oil. Five experimental crushing processes were tested in the oil-mill pilot plant of CREOL (Pessac-F) in comparison with the traditional crushing process (flaking-cooking-pressing-classical solvent extraction [C]). The five treatments were : cooking before flaking (135°C during 60 min and 135°C during 80 min, cooking before pressing (105°C), pressing and classical solvent extraction [CBF60 and CBF80], cold pressing, extrusion (two values for the die-wormshaft spacing) and classical solvent extraction [PE1 and PE2], flaking-cooking-pressing-long term solvent extraction [LTD]. The treatments CBF60, CBF80, PE1 and PE2 highly decreased protein solubility of deoiled meal (respectively 24%, 22%, 33% and 28%, as measured by the solubility in NaOH), the solubility of the control being 45%. The ruminal degradability of meals obtained by treatments CBF80 and PE2 were 55% vs 63% for the control and 30% in the case of a formaldehyde treatment. Nevertheless, the quality of crude oil, as measured by peroxide and para-anisidine index, was altered, probably due to heat treatments. The LTD treatment (heat treatment on deoiled meal) is probably the best way to protect the meal proteins (solubility in NaOH of 20%) while preserving the quality of the crude oil. Further studies are actually done in the pilot plant of CREOL for optimizing the parameters of desolventation.

Introduction

High producing animals (dairy cows) need essential amino-acids (lysine, methionine) at the small intestine. These amino-acids are not synthesised in sufficient amounts by the rumen micro-organisms (Vérité et Peyraud, 1988) and must be supplemented under the form of protected protein able to be transferred through the rumen without degradation (by-pass effect). Rapeseed meal is appreciated in animal feeding because of its high content of protein and the well balanced amino-acid composition. Associated in a diet, with grass silage containing highly degradable proteins, the rapeseed meal proteins will be optimally utilized if they are able to by-pass the rumen. Several processes were studied to lower the degradability of the protein and currently, the more efficient consists in a chemical reaction with formaldehyde. This treatment, called tanning, has been carried out industrially, for more than twenty years, on the oil cakes of rapeseed and soybean. The tanning by formaldehyde currently used in France (INRA, 1992) is effective since the degradability is lowered from 69 % to 30 % (for rapeseed meal proteins) and the increase of the quality allows its payback. The major part (60%) of rapeseed meal actually used in France by dairy cows is treated by formaldehyde. However, questions about risks of formaldehyde for consumers have been recently raised and this treatment, even accepted by the French agency for food safety (AFSSA, 2004), remains potentially suspect because of its chemical nature and the hazardous handling during the process. On the assumption of a future limitation of formaldehyde by the European rules, alternative ways avoiding chemicals could be then proposed. A few years ago, such processes based on thermal treatment have been experimented on seeds (Expro process), flakes or meal (Herlam, 1996). Heating before the extraction steps may have significant effects on the quality of the oil. The phospholipase-D enzymes could be inhibited and the phospholipids content of the oil, modified. In addition, an oxidation of the oil may occur. These effects have to be precisely evaluated to determine the nutritional and economical interest of the thermal tanning processes compared to the current practice.

In the present work, five crushing processes have been studied in comparison with the traditional crushing process, in the oil-mill pilot plant of CREOL (Pessac-F). These processes involved a heating treatment (cooking or extrusion) before, between or after the pressing and solvent extraction steps. Two technical criteria were evaluated: degradability of rapeseed proteins measured *in sacco* on fistulated cows, quality of the crude oil (peroxide and para-anisidine indexes, phosphorus content).

Experimental

Material **:** the different batches of rapeseed seeds used for the experiments were produced in France in 2003 in the Charentes-Poitou area. The cultivars were not identified, but the quality of the seeds was in agreement with the national trade rules (moisture, impurities and glucosinolates).

Technological treatments : the classical process used in industry and the five processes studied are described in the figure 1. The classical process C consisted in flaking at 300 kg/h (Flaker Damman-Croes with distance between rolls : 0,1 mm), cooking (vertical cooker heated by jacket steam, 95 °C, 60 min), pressing at 300 kg/h (Press Mécanique Moderne model MBU 75), continuous solvent extraction (Belt extractor De Smet, 50-55 °C; flow rates cakes and solvent : 130 kg/h and 220 l/h) and desolventisation (Desolventiser-toaster De Smet, Schumacher type, steam injection : 20 kg/h, temperature lower plate : 110 °C, duration 90 min). The miscella was distilled in a continuous three stages stainless-steel distiller (De Smet, at 90°C and vacuum in the stages at 400 hPa, 200 hPa and 45 hPa).

Two processes, CBF60 and CBF80 (cooking before flaking), used the heating treatment before the oil extraction. The heating was carried out by cooking the seeds at high temperature (135 °C) during 60 min and 80 min before flaking. The flow-rate was 300 kg/h. The flakes were then kept at 105 °C during 10 min, then pressed, extracted by solvent and desolventised following the classical way.

Two other processes, PE1 and PE2 (pressing-extrusion), used the heating treatment after the cold pressing and before the solvent extraction of the oil. The seeds were pressed at 300 kg/h and the cake was then extruded at 200 kg/h. The die-wormshaft spacing of the extruder (France-Extrusion) was adjusted to give different temperatures (135 °C for PE1 and 162 °C for PE2). The extruded cakes were then extracted by solvent and desolventised following the classical way.

The process LTD (long-term desolventisation) followed the classical way, except for the desolventisation step. The temperature in the desolventised-toaster (DT) was like in industry (110 °C) but the duration of the treatment was increased until 4 h by stopping the flow of matter in the DT.

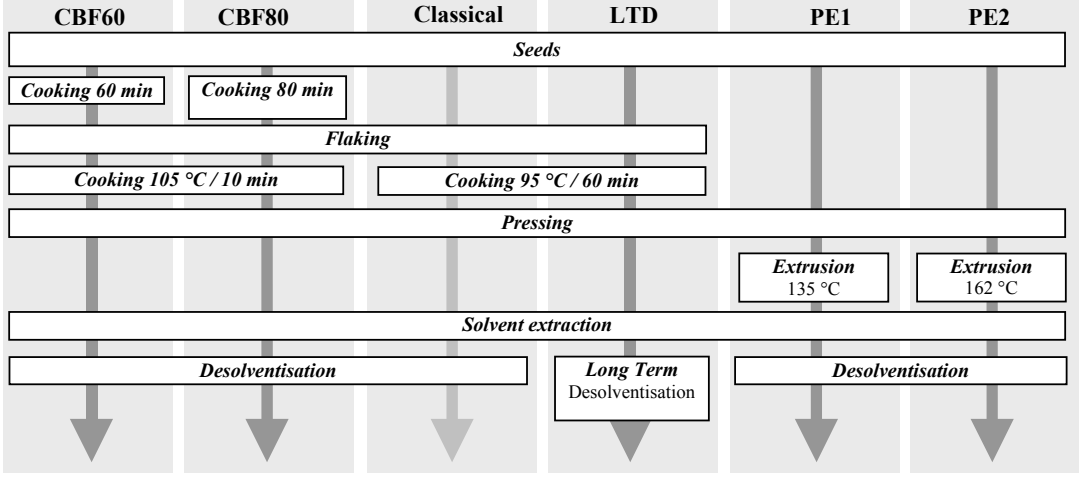

Figure 1: the different processes studied in the CREOL pilot plant, for tanning rapeseed proteins.

Analysis of meal and seed : standardized methods were used to determine the content of oil (ISO 659 and ISO 10565), moisture (V03-909), protein (NF V18-120 and ISO 5983), glucosinolates (ISO 9167-1). In-house validated methods were used for protein solubility in NaOH, enzymatic digestibility 1 h (DE1). The theoretical degradability (Dth) was determined *in sacco* (Nylon bag method) on non-lactating cows (Holstein) equipped with a rumen canula.

Analysis of oil : as the heating treatment of CBF60, classical and processes occurred before pressing, the oil quality was determined on the press oils. On opposite, for PE2 process, the impact of the heating treatment was determined after the extrusion step, on the oil obtained by pressing the extruded cake. Oil quality was not measured with LTD, CBF80 and PE1 processes.

Standardized methods were used to determine the oleic acidity (ISO 660), peroxide index (ISO 3960) and p-anisidine index (ISO 6885). Phosphorus was determined according to IUPAC 2.423 before and after degumming with 3 % water at 75 °C during 30 min and centrifugal separation.

Results and discussion

Cooking Before Flaking (CBF) and Pressing Extrusion (PE) Processes

The materials processed by CBF and PE (seeds or expeller meal) have an important content of oil. As the processes used a heat treatment (cooking or extrusion) for tanning the proteins, which may have a detrimental effect on the oil quality, the determination of the quality of both meal and oil was then necessary (table 1).

Quality of the de-oiled meals : processes CBF60, CBF80, PE1 and PE2 decreased protein solubility (in NaOH), from 85 % for the seeds to respectively to 24, 22, 33 and 28 % for the de-oiled meals. For the Classical process, the decreasing is lower: from 85 % to 45 %. The contribution of the tanning step in each process can be observed in figure 2, by comparing the effect of the treatment before the solvent extraction. Results show logically, that CBF80 is more efficient than CBF60, and PE2 than PE1. In addition, lower is the solubility of the proteins of the meal before the solvent extraction, weaker is the effect of the classical desolventisation treatment.

The theoretical degradability (Dth) was only measured on BCF80, PE1 and the Classical meal. Compared to the

Classical meal, the PDIA value (content of protein which by-passes the rumen) of CBF80 and PE1 meals were increased by respectively 27 % (from 122 to 155 g/kg DM) and 17 % (from 122 to 143 g/kg DM). These results must to be compared to the tanning effect obtained by the action of formaldehyde. The Dth of a rapeseed meal tanned with formaldehyde is around 30 % and the PDIA value is 212 g/kg DM (INRA-AFZ, 2002). The efficiency of the heat tanning is then lower than the chemical one. The levels of hexane residue in the meals BCF60, BCF80, PE1 and PE2 are significantly lower than in the "Classical" meal. In addition, compared to the classical process, only PE1 and PE2 allowed a better extraction of the oil (1,6 % versus 2,3 %). These results could be explained by the beneficial effects of the cooking and extrusion treatment on the extractability (rate of extraction under specific conditions) and the inextractible oil content and by the low moisture content of the cakes before extraction by solvent.

Table 1: Quality of the oils and meals obtained par the processes CBF60, CBF 80, Classical, PE1 and PE2.

Process		CBF60	CBF80	Classical	PE1	PE2
Quality of seeds						
Oil content (% DM)		48,9	48,9	48,9	48,9	
Protein solubility in NaOH (%)		85	85	85	85	
Quality of expeller cakes						
Cooking before pressing		130 °C/ 60 min	130 °C/ 80 min	95 °C/ 60 min	no	
Moisture content (%)		4,4	4,0	7,0	8,3	
Oil content (% DM)		17,2	16,6	15,0	18,4	
Protein solubility in NaOH (%)		35	29	75	86	
Quality of the press oil						
Oleic acidity (%)		0,39	nd	0,46	0,19	
Peroxide index ($meqO_2$/kg) (IP)		2,2	nd	2,8	1,9	
p-anisidine index (Ian)		4,8	nd	1,4	1,8	
Tot-Ox Index (Ian + 2 IP)		9,2	nd	7,0	5,6	
Phosphorus (mg/kg)	Before degumming	177	nd	351	15	
	After degumming	6	nd	302	5	
Quality of the extruded flakes						
Extrusion conditions		no	no	no	135 °C	162 °C
Moisture content (%)		nd	nd	nd	4,2	3,3
Oil content (% DM)		nd	nd	nd	18,0	19,2
Protein solubility in NaOH (%)		nd	nd	nd	48	42
Quality of the residual oil *						
Oleic acidity (%)		nd	nd	nd	nd	3,3
Peroxide index ($meqO_2$/kg) (IP)		nd	nd	nd	nd	1,7
p-anisidine index (Ian)		nd	nd	nd	nd	18,1
Tot-Ox Index (Ian + 2 IP)		nd	nd	nd	nd	21,5
Phosphorus (mg/kg)	Before degumming	nd	nd	nd	nd	3110
	After degumming	nd	nd	nd	nd	50
Quality of the de-oiled meal						
Moisture content (%)		7,9	8,0	8,5	9,6	8,2
Oil content (% DM)		2,0	2,3	2,3	1,6	1,6
Hexane residue (mg/kg)		105	108	494	176	182
Protein solubility in NaOH (%)		24	22	45	33	28
Enzymatic digestibility (%)		nd	14,0	31,0	14,8	nd
Theoretical degradability in sacco (%)		nd	55,3	63,2	55,5	nd
PDIA (g/kg DM)		nd	155	122	143	nd

nd : no determined PDIA : proteins digestible in intestine * obtained by pressing

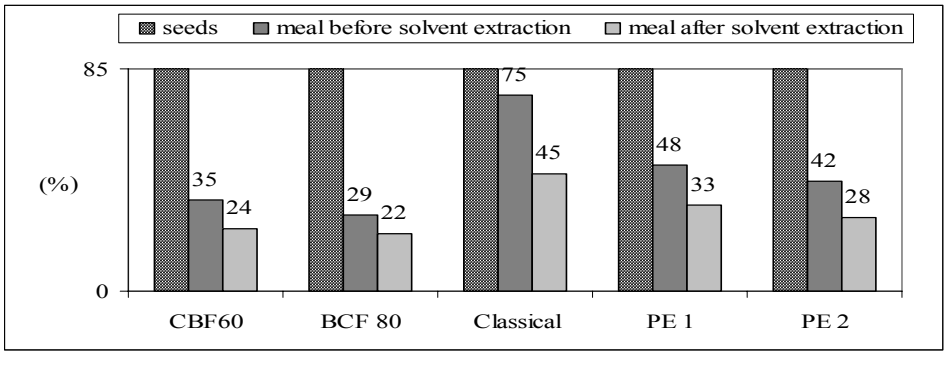

Figure 2: Solubility of proteins (in soda) measured at different steps of the five processes.

Quality of the oil : The effects of the thermal treatments can be checked (table 1) on the Tot-Ox index of the press oil which increases from 5,6 for the PE1 oil (cold pressing) to 7,0 for the "classical" oil (cooking at 95 °C and pressing) and 9,2 for the CBF60 oil (cooking at 130 °C and pressing). The level of non-hydratable phospholipids is low in the CBF60 press oil (6 mg/kg) because of the inactivation of the phospholipases-D during the cooking. With the classical process, the phosphorus content is high (350 mg/kg) and the main part (86 %) is in the non-hydratable form. For the PE process, the press oil obtained before extrusion has a lox Tot-Ox value (< 6) and a low total phosphorus content (15 mg/kg) as it is usual with cold pressing oils. In contrast, the quality of the oil obtained (by pressing) after the cooking-extrusion treatment has dramatically decreased since Tot-Ox value is more than 20 and the content of total phosphorus is very high (> 3000 mg/kg).

Long-Term Desolventisation Process (LTD)

Previous experiments to optimise the tanning during the desolventisation showed that the high temperature (> 125 °C) necessary to obtain a significant effect, was difficult to apply in the DT because of the high pressure of steam necessary (1 MPa). In addition, the control of the duration of the treatment in a continuous flow was found difficult because of the geometry of the DT (important dead volumes). To apply a heat treatment, the alternative way to high temperature, was to maintain the usual temperature (110 °C), to stop the steam injection, and to increase the duration of the presence of the meal in the DT. The supposed beneficial is the saving of energy and a better control of the treatment duration.

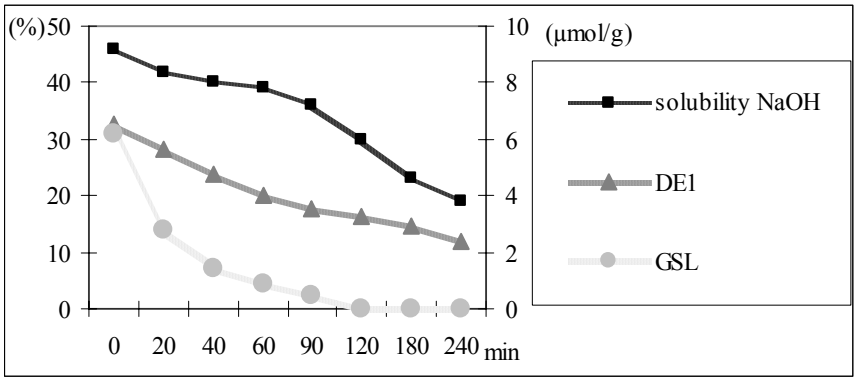

Figure 3: Decrease of the solubility (in NaOH) of the proteins, of the enzymatic digestibility 1 hour (DE1) and the glucosinolates (GSL) content of rapeseed meal in the DT at 110 °C without steam injection.

Figure 3 shows preliminary results on the" response" of the meal when treated during a long time in the DT. The solubility of the proteins, the DE1 value and the glucosinolates (GSL) content was decreasing during four hours. These results were obtained by stopping the flow of the matter and their reproducibility indicates that the optimisation of the process to reach the control of the tanning effect, the breakdown of the GSL and the hexane residue will be more efficient on equipment working in batch than on a continuous flow. Then, our future experiments on this process will be carried out on a small sized reactor simulating the desolventisation.

Conclusion

This study showed that heating rapeseed for tanning the proteins is accompanied by an oxidation of the oil. Such processes applied on seeds or expeller meals in a way to reduce significantly the theoretical degradability of the proteins, produce press or solvent extraction oil with an alteration which may not be reduced by refining. On opposite, the heating treatment may have a beneficial effect on the phosphorus content and the nature, hydratable or not, of the phospholipids.

These results direct our research on the way of the long-term desolventisation (LTD) because this process does not involve the heating of the non extracted oil. This process may also save energy since, the heat absorbed by the meal does not need to be increased but only maintained for a longer period. Moreover, the equipment involved is less expensive than an extruder or a cooker and could be easily applied in industrial plants.

The optimisation of the LTD process will be possible only if the experimental conditions are well controlled. Our experience with a pilot-scale DT shows that a laboratory scale equipment is necessary to better control the conditions, to get numerous samples of meal (especially when non desolventised), to monitor kinetics and to establish a model for simulation. The pilot-scale experiment will confirm and allow the extrapolation of the results.

References

AFSSA, 2004. Evaluation des risques liés à l'utilisation du formaldéhyde en alimentation animale. 25 p.

INRA, 1992. Procédé de traitement de matières protéiques, produits obtenus et application en alimentation animale. Brevet 2129.

Herlam, J., 1996. Heat treated Rapeseed Meal for Dairy Cows. Fett/Lipid. 98, N° 7/8, 246-249.

Vérité R. and Peyraud J.-L.,1988. Nutrition azotée. *In* Alimentation des bovins, ovins & caprins. Ed. R. Jarrigé., INRA, Paris. 75-93.

INRA-AFZ, 2002. Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. Ed. D. Sauvant, J.-M. Perez, G. Tran. INRA, Paris, 301 p.

The advancement of double-low rapeseed meal used as a protein feedstuff in pig and poultry diets

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Abstract

The article reviews the nutritional characteristics and the potential factors that negatively affect the extensive use of double-low rapeseed meals (DLRM) as a protein source in animal diets, and discusses the feasibility to improve the feeding values of DLRM-containing diets by dietary enzyme supplements and/or hull removal treatment by processing techniques. Overall, crude protein content of DLRM is comparable with soybean meal. Also, DLRM has a well balanced amino acid profile and, consequently, a diet with more balanced amino acid profile can be obtained by the combination use of DLRM with soybean meal. Similar even better performance was observed in pigs fed conventional level of DLRM diets (⩽ 6% and 10% of diet for the growing and finishing phase, respectively). However, the unrestricted use of DLRM in rapid growth animals was limited by low available energy resulting from the high content of fibres. Hull removal could reduce the content of insoluble fibres like lignin, and thus improve the digestible energy and crude protein levels, whereas the total non-starch polysaccharides remained same as that in not dehulled DLRM. Decreased weight gain was evidenced in pigs receiving diets incorporated with 10~15% of dehulled DLRM, and in broiler chickens receiving diets incorporated with 21% of dehulled DLRM. Enzyme supplementation could result in improved performance and increase the inclusion levels of DLRM to 10~15% in pigs and 20~23.5% in broilers. It would appear that enhanced nutritional values and, consequently, increased inclusion levels of DLRM in animal diets could be achieved by the combination use of enzyme supplements with hull removal of DLRM by processing techniques.

Key words: Double-low rapeseed meal, hull removal, enzyme supplementation

Introduction

Double-low rapeseed (referred to as a rapeseed cultivar that contains less than 2% erucic acid in its oil and less than 30 μmol/g of glucosinolates in its defatted meal), more commonly known as canola, is second only to soybean as the most important source of vegetable oil in the world. After oil extraction, the remaining part of the seed is known as canola meal (CM) or Doule-low rapeseed meal (DLRM). The high levels of protein and the good balance of essential amino acids make the meal especially valuable as a protein supplement in feed rations for livestock. Despite its benefits, the inclusion levels of CM in monogastric animals is still limited, which is mainly caused by the high levels of fibres (Bell, 1993). Hull removal of canola and the supplementation of enzyme in CM-containing diets are shown to be potential methods to improve the nutritive value of CM. The goal of this paper is to review the nutritional characteristics and the potential factors that negatively affect the extensive use of CM as a protein source in animal diets, and discuss the feasibility to improve the feeding values of CM-containing diets by dietary enzyme supplements and/or hull removal treatment by processing techniques.

The chemical components of CM and its use in animal feed

CM is lower in crude protein than soybean meal (NRC, 1998; Table 1). CM contains approximately 36 percent crude protein compared to 44 percent crude protein for soybean meal. Interestingly, Chinese DLRM has higher protein content than CM and is comparable with soybean meal. Both CM and Chinese DLRM have approximately twice content of calcium and phosphorus than soybean meal. However, the content of fibers such as NDF and ADF is higher in CM than in soybean meal, which was considered to be the major factor resulting in a low digestible energy of CM. Remarkably, Chinese DLRM has much higher fiber content than CM and soybean meal. Research conducted at Huazhong Agricultural University revealed that the much higher fibre content of Chinese DLRM compared to CM was caused by over-heat treatment (Peng, 2000). In particularly, the increase of cell wall protein content in the over-heat treated samples were responsible the large enhancement of dietary fibre. Subsequently, Chen et al. (2003, 2006) investigated the processing on the quality of Chinese DLRM with the focus on the NDF content. The research found that the increment of NDF always occurred in the stages of cooking/conditioning, pressing, dissolventing where heat treatment was adopted (Table 2). In these stages, considerable amount of protein turned to products of maillard reaction. Therefore, to control the quality of rapeseed meals and cakes, NDF content has been regarded as a valid indicator in the current rapeseed-processing conditions in China.

Maybe, just because the difference in nutritive profiles between CM and Chinese DLRM, varied inclusion levels of these two types of meals was proposed in monogastric animal feeds. Thacker (1990) concluded that CM might replace 50% of the protein source in grower and finisher diets without any possible detrimental effect on production. Brand et al. (2001) reported that there was no effect on production performance of pigs receiving diets with solvent-extracted CM up to 24%, and no effect with expeller-extracted CM up to 29.2%. These inclusion rates are much higher than inclusion levels of 12% in diets of growing pigs and 18% in diets of finishing pigs proposed by the Canola Council of Canada (1989). It may be concluded that

the meal processed from recently released canola cultivars, which contain lower levels of anti-nutritional factors (Mailer & Colton 1995), are more acceptable for growing finishing pigs and may be included at higher levels. In contrast, the research conducted in China found that the appropriate addition levels of Chinese DLRM were ⩽6% in the growing phase and ⩽10% in the finishing phase, respectively (Peng et al., 1995). The digestibility and metabolism trial conducted by Peng (2000) provide further evidence for the quality difference between CM and Chinese DLRM. TMEn for three Chinese DLRM samples in SCWL cockerels was 6088-6095 KJ/kg, significantly lower than 8327 KJ/kg for commercial CM. Apparent total-tract digestibility and true ileal digestibility of crude protein in pigs were 67.05-70.12% and 53.44-63.65%, respectively, for three Chinese DLRM samples, substantially lower than 73.61% and 68.95%, respectively, for commercial CM. Notably, a better performance could be obtained when DLRM containing diet for pigs were formulated based on the digestible lysine levels (Peng et al., 1999). This could be explained by the low availability of lysine in Chinese DLRM, whereas sulfur amino acids are particularly higher in canola than in soybean meal and, consequently, a diet with more balanced amino acid profile could be obtained when CM and soybean meal are in combination use.

Table 1. Nutrient Analysis of CM Compared to Soybean Meal (%)

Nutrient	Soybean Meal[1]	CM[1]	Chinese DLRM[2]
CP	43.8	35.6	42.5
NDF	13.3	21.2	45.0
ADF	9.4	17.2	30.9
Calcium	0.32	0.63	0.75
Phosphorus	0.65	1.01	1.03

[1]Data adapted from "Nutrient Requirements of Swine", 10th Ed. (1998).
[2]Data adapted from **"**Evaluation and Improvement of Quality of Chinese Double Low Rapeseed Meal", Peng (2000)**.**

Table 2. NDF content and fraction of protein contained in NDF in samples from different processing stage (% dry matter, fat free)[1]

Processing stage	Sample type	Low-temperature-press		Prepress-extraction		Hydraulic-press	
		Mean	Range	Mean	Range	Mean	Range
Raw material	Seed	32.8 (6.3)	30.5~34.2 (5.7~7.3)	34.0 (6.4)	32.8~35.6 (5.5~7.8)	34.5 (5.8)	33.0~35.4 (5.1~7.3)
Flaking	Precooked seed	-	-	32.1 (6.2)	30.0~33.1 (5.3~8.1)	-	-
Cooking/conditioning	Cooked seed	-	-	33.1 (12.8)	31.0~34.5 (9.7~15.8)	46.3 (31.7)	39.4~54.2 (27.2~34.5)
Expelling	Prepressing cake	30.5 (6.0)	28.7~31.4 (5.4~6.7)	31.3 (12.7)	29.1~33.6 (8.7~15.4)	55.8 (45.1)	48.9~59.8 (37.6~47.4)
Dissolventing	Meal	-	-	37.8 (19.1)	33.8~45.6 (15.8~24.8)	-	-

[1]Data adapted from **"**Evaluation of quality characters, quality influencing factors and processing technics of Chinese Rapeseed cake and meal", Cheng (2003)**.**

The routinely use of CM in poultry diets can be ascribed to two aspects: first, the amino acid pattern resembles that of the ideal protein proposed for poultry (Baker and Chung, 1992), and second, CM has high contents of arginine and sulfur amino acid, which is particularly deficiency in conventional corn-soya diet. Liu et al. (2004) indicated that the appropriate inclusion levels of Chinese DLRM in broiler chickens, expressed as the percentage of DLRM protein to total dietary protein derived from soybean meal and Chinese DLRM, were 37.5%-50% for 0-21 days of age and 50%-62.5% for 21-42 days of age. In laying hen diet, inclusion of 15-20% of diet was considered to be allowable considering that these inclusion levels could not result in negative effect on egg production or feed efficiency (Trappett, 2001; Roth-Maler,1999). However, the inclusion level of CM in brown egg-hen was recommended to be 3-5% of diet, due to the presence of considerable amount of choline and sinapine, which was the precursor of trimethylamine. It has been well established that feedstuffs rich in trimethylamine could result in fishy smell eggs (Butler et., 1982).

Effect of hull removal on the nutritional values of CM

Canola hull constitutes about 16%-19% of the seed and about 25%-30% of the meal (Bell, 1993), the hull fraction has a very low digestibility. Therefore, hull removal was considered to be one option available to improve the digestibility of energy in CM. Zuprizal et al.(1992) reported that hull removal could reduce crude fibre from 13.3% of meal to 6.6% (DM basis) and increase protein digestibility from 70.5 to 76.7% in 6 week broilers, and average amino acid digestibilities from 80.8 to 85.2% in ISA Brown roosters (Zuprizal et al. 1991). It was shown in pig trials that partial dehulling increased the digestible energy (DE) content from 12.2 to 13.3 MJ kg DM^{-1}, respectively, and the level of crude protein (CP) from 40.6 to 43.8% in regular and partially dehulled CM samples, respectively (de Lange et al., 1998). However, hull removal did not influence apparent ileal CP or AA digestibilities, except for threonine which was slightly increased.

Kracht et al. (1999) compared the influence of graded rapeseed meal levels (7%, 14%, 21%) from hulled and dehulled rapeseed on growth performance and found that the in the average of the three levels the weight gain of broilers fed dehulled rapeseed meal diets rose about 53 g (=3.5%) compared with that fed hulled rapeseed meal diets although at a substitution level of 21% the growth decreased. In contrast, Campbell et al. (1995) using dehulled CM to replace hulled CM incorporated into broilers' or laying hens' diets didn't result in increased growth rate or improved laying performance. However, broilers responded to lysine supplementation in the dehulled meal treatments which corroborates the amino acid analysis data for the

respective. Remarkably, hens fed dehulled CMs tended to show a reduced egg production rate. This effect may have been due to a relatively high glucosinolate content in the dehulled meal samples (Campbell et al., 1995).

In contrast to poultry, study reports about the growth response of pigs to dehulled CM inclusion were relatively scarce. Bell (1993) compared the effect of dehulled CM to replace for hulled CM in growing and finishing pig diets and observed no improvement in feed efficiency. Similarly, Patience and Gillis (1996) reported that pigs receiving diets containing 15% (growing phase, 24 - 56 kg) or 10% (finishing phase, 71 - 100 kg) dehulled CM had a similar growth rate and feed efficiency compared to those receiving hulled CM diet. The modest response may be explained by the following aspects: the reduction in dietary fibre following hull removal was mainly reflected by a decrease in insoluble fibre, lignin in particular, but total non-starch polysaccharides (NSP) still accounts for some 17.8-21.4%, as near as making no difference from that present in hulled CM (16-22%) (Campbell et al., 1995); at the same time, hull removal may cause an increased level of soluble fibre and worse viscosity problem for that a majority of soluble fibre is present in cotyledon of CM (Peng, 2001). In conclusion, the moderate levels at which CM is used in pig diets, combined with the modest improvement in pig performance, make the economics of dehulling questionable.

Potential benefits of enzyme supplementation in CM diet

It was reported that the levels of starch, free sugars and soluble NSP in CM is about 150 g/kg, which should contribute to considerable digestible energy (Slominski and Campbell, 1990). Unfortunately, it appears that these carbohydrates are encapsulated by cell walls and that their actual contribution to digestible energy is modest (Bell, 1993). Enzyme supplementation is thus considered as a potential means of improving the nutrient digestibility and ME of CM. More than 15 years ago, a number of studies had been conducted to investigate the response of broilers to enzyme supplementation in CM diet. Most feeding trials have indicated that enzyme supplementation of CM with carbohydrase and protease preparations does not produce a statistically significant improvement in broiler chick performance (Simbaya et al. 1996; Alloui et al. 1994; Sosulski et al., 1990), although results to the contrary exist (Ward et al. 1991; Bedford and Morgan, 1995). These results demonstrated the difficulty to find appropriate enzymes to match the specific substrate present in CM, for that CM as a protein source has only a small amount of inclusion in animal diet. In the latest decade, Canadian University of Manitoba undertook a series of studies to select enzymes effective in improving canola by in vitro and in vivo methods. Slominski and his co-workers recently demonstrated both an improved broiler performance and an increased nutrient utilization as a result of enhanced depolymerization of cell wall polysaccharides by multicarbohydrase supplementation in CM diets (Meng and Slominski, 2005). In addition, phytase supplementation of CM-based rations (500 g kg^{-1}) was shown to improve phosphorus and calcium retention in broilers aged 7-14 days (Ward *et al.* 1991). The best response of chicks to phytase supplementation was found when phytase was fed in combination with protease (Guenter et al., 1998).

In China, Peng and her co-workers have been taking up researches on canola feeding value and its improvement in the last 15 years. The appropriate inclusion levels of Chinese DLRM in pig diets were determined to be 6-10% (Peng et al., 1995; 1999), substantially lower than the proposed levels of CM (12-18%) by Canola Council of Canada (1989). A comprehensive evaluation of Chinese DLRM nutrient profiles revealed that it was the much higher fibre content that makes Chinese DLRM superior to CM regarding their feeding values (Peng, 2000). Peng (2000) confirmed that Chinese DLRM has similar fibre component compared to CM. The fibre components of CM include lignin with associated polyphenols (8%), cellulose (4-6%) and non-cellulosic polysaccharides (13-16%) which consists of arabinose (33%), xylose (13%), mannose (3%), rhamnose (2%), fucose (2%), uronic acids (30%), galactose (13%) and glucose (5%) (Slominski and Campbell, 1990). The high content of arabinose and xylose in DLRM indicated the presence of considerable amount of arabinoxylans (Slominski and Campbell, 1990). In this regard, xylan-related substrates may play a major role in negatively affecting the nutritional values of DLRM (Fang et al., 2006). Furthermore, previous evidence has demonstrated the effectiveness of xylanase supplementation in improving the growth performance of broilers (Bedford and Morgan, 1995) fed DLRM inclusion diets. Therefore, Peng and her co-workers conducted a series of researches to select xylanase-based enzymes targeting Chinese DLRM by in vivo and in vitro methods. The two Master's Degree thesis in Huazhong Agricultural University provide a comprehensive demonstration about the selection of enzymes and the efficacy of selected enzymes in improving Chinese DLRM (Tang, 2003; Fang, 2005). Recent trials show that the inclusion levels of Chinese DLRM can be enhanced from the conventional 6-10% in pigs and 10-15% in broilers to 10-15% in pigs (Tang, 2006) and 20~23.5% in broilers (Fang, 2005), respectively, by selected enzyme supplementation.

Summary

The low digestible energy is the major restriction to the expanded use of CM in rapidly growing animals. High content of fibre, cell wall protein in particular, was the main difference in nutrient profiles between CM and Chinese DLRM. Hull removal can be effective in enhancing digestible energy and crude protein contents, but should be cautioned taking into account its economics. Alternatively, enzyme supplementation is considered to have substantial potential to improve CM or Chinese DLRM.

References

Alloui O., Chibowska M., Smulikowska S. (1994). Effects of enzyme supplementation on the digestion of low glucosinolate rapeseed meal in vitro, and its utilization by broiler chicks. Journal of Animal and Feed Sciences 3, 119-128.

Baker H.D., Chung T.K. (1992). Ideal protein for swine and poultry. Biokyowa Technical Review, 4.

Bedford M.R., Morgan A.J. (1995). The use of enzymes in canola–based diets. In: W. van Hartingsveldt, M. Hessing, J. P. van der Lugt, W. A. C. Somers (eds),

2nd European Symposium on feed enzymes. Proceedings of ESFE2, Noordwijkerhout, The Netherlands, 125-131.
Bell J.M. (1993). Factors affecting the nutritional value of canola meals: A review. Canadian Journal of Animal Science **73**, 679-697.
Brand T.S., Brandt D.A., Cruywagen C.W. (2001). Utilisation of growing-finishing pig diets containing high levels of solvent or expeller oil extracted canola meal. New Zealand Journal of Agricultural Research **44**, 31-35.
Butler E.J., Pearson A.W., Fenwick G.R. (1982). Problems which limit the use of rapeseed meal as a protein source in poultry diets. Journal of the Science of Food and Agricuture **33**, 866-875.
Campbell L.D., Simbaya J., Zhang W., Slominski B.A., Guenter W. (1995). Nutritive value of dehulled canola meal. Pages 179-181 in Proceedings of the 9th International Rapeseed Congress. Cambridge, UK.
Canola Council of Canada. (1989). Canadian canola meal: Maximum inclusion rates. Winnipeg, Canada, Canola Council of Canada.
Chen G. (2003). Evaluation of quality characteristics and its influencing factors of Chinese rapeseed cakes and rapeseed meals. M.D. Diss., Huazhong Agric. Univ., Wuhan, China.
De Lange, C.F.M., Gabert V.M., Gillis D. Patience. J.F. (1998). Digestible energy contents and apparent ileal amino acid digestibilities in regular or partial mechanically dehulled canola meal samples fed to growing pigs. Canadian Journal of Animal Science **78**, 641-648.
Fang Z.F. (2005). Screening of special cocktail enzyme for Chinese canola meal based diet In Vitro and its application to pigs and poultry. M.D. Diss., Huazhong Agric. Univ., Wuhan, Hubei.
Fang Z.F., Peng J., Liu Z.L., Liu Y.G. (2006). Responses of non-starch polysaccharide-degrading enzymes on digestibility and performance of growing pigs fed a diet based on corn, soybean meal and Chinese double-low rapeseed meal. Journal of Animal Physiology and Animal Nutrition. In press.
Guenter W., Slominski B.A., Campbell L.D. (1998). Enhancement of the feeding value of canola meals using exogenous enzymes. Canola Utilization Assistance Program, Research Report No. #94-10/93-21C. 45-49.
Kracht W., Jeroch H., Daenicke S., Matzke W. (1999). Effect of dehulling rapeseed on feed value of rapeseed meal and cake for poultry. In Proceedings of the 10th international rapeseed congress, Canberra, Australia.
Liu Z.L., Peng J., Fan H.Y., Tang B. (2004). Research on the appropriate addition levels of double-low rapeseed meal in broiler diets. Feed Industry **25**, 29-31.
Mailer R.J., Colton R.T. (1995). Quality of Australian canola. Vol. 3. Canola Association of Australia, Canola Research Laboratories, Agricultural Research Institute, Wagga Wagga, NSW, Australia.
Meng X. Slominski, B.A. (2005). Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a muticarbohydrase preparation of cell wall degrading enzymes. Poultry Science **84,**1242-1251.
NRC. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.
Patience J.F., Gillis D. (1996). The Evaluation of Dehulled Canola Meal in the Diets of Growing and Finishing Pigs. Prairie Swine Center INC. 1996 Annual Research Report.35-39
Peng J. (1995). The experiment of substituting Chinese canola meal for soybean meal on equal nitrogen basis in diets for growing-finishing pigs. China Feed. (24), 13-16.
Peng J. (2000). Evaluation and improvement of quality of Chinese double-low rapeseed meal. Ph.D. Diss., Huazhong Agric. Univ., Wuhan, Hubei.
Peng J., Slominski B.A., Guenter W., Campbell L.D., Xiong, Y.Z. (2001). The antinutritional factors in Chinese canola meal. Journal of the Chinese Cereals and Oils Association **16** (5), 6-9.
Roth-Maier D.A. (1999). Investigations on feeding full-fat canola seed and canola meal to poultry. Proceedings 10th International Rapeseed Congress, Canberra, Australia.
Simbaya J., Slominski B.A., Guenter W., Morgan A., Campbell L.D. (1996). The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: In vitro and in vivo studies. Anim. Feed Sci. Technol **61**, 219-234.
Slominski B.A., Campbell L.D. (1990). Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. Journal of the Science of Food and Agriculture **53**, 175-184.
Sosulski K., Sosulski F., Thacker P. (1990). Oil quality, chemical characteristics and nutritional quality of presscakes from enzyme treated canola. Research on canola meal. An excerpt from the 10th project report. Canola Council of Canada. 44-63.
Tang T.J. (2003). Research of the enzyme additives for non-starch polysaccharides applied to the double-low rapeseed meal, rice and wheat basal diets in vitro and in vivo. M.D. Diss., Huazhong Agric. Univ., Wuhan, China.
Tang T.J. (2006). Effects of enzyme additives in wheat- and double-low rapeseed meal-based deits on performance of growing-finishing pigs. Journal of Huazhong Agricultural. University **25**, 159-163.
Thacker P.A. (1990). Canola meal. In: Thacker, P. A.;Kirkwood, R. N. ed. Non-traditional feed sources for use in swine production. Saskatchewan, Canada, Department of Animal and Poultry Science, University of Saskatchewan. 69-78.
Trappett P. (2001). Low glucosinolate canola meals for laying hens. Cited by W.A. Dudley Cash in Feedstuffs, May 7, 12.
Ward A.T., Rotter B., Thacker P.A., Campbell G.L. (1991). The effect of enzymes on the availability of minerals and on the growth of chicks fed canola based diets. Research on canola meal. The 11th project report. Canola Council of Canada. 84-87.
Zuprizal, Larbier, M., Chagneau A.M., Lessire M. (1991). Effect of protein intake on true digestibility of amino acids in rapeseed meals for adult roosters force fed with moistened feed. Animal. Feed Science and Technology **34,** 255-260.
Zuprizal, Larbier M., Chagneau A.M. (1992). Effect of age and sex on true digestibility of amino acids of rapeseed and soybean meals in growing broilers. Poultry Science **71**, 1486-1492.

The effect of canola meals on the performance of broiler chicks

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Abstract

In order to study the effect of replacing different levels of canola meals to soybean meals (0, 5, 10, 15 and 20 percent) on broiler performance with 3 replicates a randomized complete design were used. Average daily gain, feed conversion were measured. At the end of the trail one male and one female of each pen were selected killed and dressing percentage, abdominal fat intestine, liver and gall bladder and pancreas weight were determined. Data from this experiment showed feed conversion and average daily gain of the group used 5 percent canola meal were better than the other groups. The best body weight related to group which percent canola meal. The highest levels of abdominal fat related to groups 15 and 20 percent canola meal used.

Introduction

Canola meal (CM) or Rape seed meal has an excellent balance of amino acids but has lower amino acid digestibility than soybean meal (SBM) (Zuprizal et al., 1992). Previous work has shown that the desolventization/toasting stage of prepress solvent extraction of canola reduces the content and digestibility of amino acids, particularly that of lysine (Newkirk and Classen, 1999; Newkirk et al., 2000). Desolventization is the process of removing the hexane used to extract the oil in a desolventizer/toaster (DT). It is called a DT because it not only desolventizes the meal but it also imparts a toasting process to reduce the level of heat-labile anti-nutritional factors in the meal. The Schumacher DT is the most prevalent design in North America and consists of a series of heated trays stacked vertically. The solvent-laden meal is conveyed onto the top tray and falls from tray to tray until exiting to a drier-cooler with low residual hexane levels remaining. The first trays are internally heated with steam, thereby heating the meal indirectly and causing the majority of the solvent to evaporate. The final trays apply direct heat in the form of sparge steam injected directly into the meal. This steam condenses and increases the moisture content of the meal exiting the DT to approximately 14.5 to 18.5%. Upon entering the DT, the solvent-extracted CM is yellow, but at exit CM is brown, indicating the Maillard reaction is occurring in the process (Newkirk and Classen, 1999). The extent of the Maillard reaction is shown by the lower lysine content and digestibility of toasted meal (Newkirk and Classen, 1999). Optimal conditions for the Maillard reaction have been defined previously in other proteinaceous products as being between 15 to 18% moisture in combination with elevated temperatures (Mauron, 1981). Because sparge steam increases the moisture content, it may contribute to losses in amino acid content and digestibility during desolventization. Therefore, elimination of additional moisture in the form of sparge steam during desolventiza-tion/toasting may result in yellow meal with an elevated concentration of amino acids and enhanced digestibility.It is not known, however, if the toasting process imparted by the sparge steam is necessary to reduce heat-labile antinutritional factors in the meal and, therefore, required to optimize the nutritional value of the meal. The objectives of the current experiment were to determine if elimination of sparge steam during desolventization would reduce the level of browning and toasting of the meal and to determine if the toasting process imparted by the sparge steam is required to reduce anti-nutritional factors and, therefore, optimize broiler performance**.**

Material and methods

240 1-d-old commercial broiler chick (Ross) were weighted, distributed randomly to 5 treatments with 3 replicates., the experiment was arranged in a randomized completely design. Four levels of rapeseed meals replacing to soybean meals including 0, 5, 10, 15 and 20 percent. Broilers were growing to 56 days of age.

Means compared with Duncan's Multiple Range Test at ($p<0.05$). The chicks were reared in deep litter on wood hulls in 24 pens 1*1. Feed and water were provided *ad -libitum.* Body weights and feed conversion. The characteristics under investigation were average daily gain, feed conversion ration, in each pen two males and females. At the end of trail one male and female of each pen were selected. The samples killed and dressing percentage heart, pancreas, liver, spleen, proventriculus, gizzard, gall bladder and intestinal weights were determined.

Results and Discussion

Feed Conversion Ratio: The results are presented in Table 2. Feed conversion data showed significant different ($p<0.05$). The results indicated that 15% and 20% rapeseed meals replacing to soybean meals for the feed conversion ratio better than the other groups. The results are in agreement with the result of other researchers (Zeb *et al.,* 1999; Summers *et al.,*1988). Most probably this is due to presence of fishmeal, keeping amino acid moderation and no exchange in anion-cation balancing at the ration. The results indicated replacing rapeseed meals 15% and 20% causes increase feed conversion ratio

because high NSP (non starch polysaccharides) or inhibitor factors in rapeseed meals.

Table1:Composition of starter diets(1-3week)

Item	Control	5%Canola	10%Canola	15%Canola	20%Canola
Corn	63.86	58.95	56.48	51.08	52.03
Soybean meal	26.97	25.83	22.76	19.7	16.65
Fish meal	6.5	6.5	6.5	6.5	6.5
Canola meal	0	5	10	15	20
Fat	0.32	1.61	2.26	2.92	3.02
DCP	0.56	0.48	0.46	0.43	0.4
Oyster shell	0.97	0.88	0.83	0.77	0.7
Mineral premix	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.11	0.05	0.02	0	0
Salt	0.2	0.2	0.2	0.2	0.2
CalculatedComposition					
ME (kcal/kg)	2950	2950	2950	2950	2950
CP(%)	21.2	21.2	21.2	21.2	21.2
Ca(%)	0.92	0.92	0.92	0.92	0.92
Total P(%)	0.41	0.41	0.41	0.41	0.41
Met+ Sys(%)	0.83	0.83	0.83	0.84	0.84
Lysine(%)	1.19	1.2	1.2	1.2	1.2

Table 2: Composition of grower diets(3-6Week)

Item	Control	5%Canola	10%Canola	15%Canola	20%Canola
Corn	70.46	64.46	64.41	62.3	53.88
Soybean meal	22.18	12.63	10.57	13.59	16.5
Fish meal	5	5	5	5	5
Canola meal	0	5	10	15	20
Fat	.05	1.09	1.63	2.19	2.7
DCP	0.65	0.41	0.32	0.29	0.3
Oyster shell	0.94	0.97	0.92	0.93	0.92
Mineral premix	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
DL-Methionine	.02	0	0	0	0
Salt	0.2	0.2	0.2	0.2	0.2
CalculatedComposition					
ME (kcal/kg)	3000	3000	3000	3000	3000
CP(%)	18.75	18.75	18.75	18.75	18.75
Ca(%)	0.84	0.84	0.84	0.84	0.84
Total P(%)	0.38	0.34	0.33	0.33	0.33
Met+ Sys(%)	0.67	0.7	0.73	0.75	0.75
Lysine (%)	1	1.01	1.02	1.03	1.04

Body weight: The results are presented in Table 3. There is no significant difference (P>0.05) from level 5% to level 15% in body weight between experimental groups in the comparison with control. The results are in agreement with the result of other researcher (Zeb *et al.,* 1999; Summers *et al.,* 1988). Most probably this is due to presence of fish meal, keeping amino acid moderation and no exchange in anion-cation balancing at the ration. Roth Maier *et al*. (1988) indicated that use of 5, 10, 15, 20 and 25 percents of full-fat Canola seed in the broiler ration has the negative effect on the chicken growth so that, body weight in experimental groups in comparison with control has showed 6.7-24% reduction. Researcher has mentioned the decreasing of feed consumption is the cause of body weight decreasing (Sosulski, 1974; Roth Maier *et al*., 1988). According to Najib and Al-Khateeb (2004) with the exception of protein level, canola seed are very much similar to canola meal. High level oil in Canola seed in comparison to its meal, will cause meal and fish meal in starter diet and low level protein ratio, had adjusted feed consumption in experimental and control groups from 1 to 21 days of study. This issue does not support some results of researchers and with some other has conformity. This is in such a manner hat it has no conformity with the results of researches (Roth Maier *et al*., 1988; Lee *et al.*, 1984 and Nassar and Arscott, 1986). Roth Maier *et al*. (1988) used 5, 10, 15, 20 and 25 full-fat canola seed in the broiler diets, has observed that increasing proportion of Canola seed in the diet reduce continuously performance. No particular cause has been reported for decreasing of feed consumption yet, but the existence of phytic acid in canola seed and meal will cause reduction in calcium ability absorption and consequently, the feed consumption reduction (Semmers *et al.*, 1988). The results of this study support other studies (Semmers *et al.,* 1988; Semmers *et al.*, 1977;

Clark *et al.*, 2001). Zeb *et al.* (1999) reported that due to securing of amino acids in ration, feed consumption will not show any reduction by adding canola meal. Also, Hill (1979) has reported. The results are presented in Table 5. body weight are shown 5% replacing canola meal better than other groups and was significant(P>0.05). Percent of carcass wasn't significant effect at the end of experiment. Percent of Abdominal fat are shown the groups 15% and 20% replacing canola meal significant effect(P>0.05) and higher than other groups. Inhibitor factors causes increase abdominal fat because inhibited basal metabolism (4). Percent of gall bladder and intestine wasn't significant effect in all groups. Percent of pancreas are shown the group 20% replacing canola meal significant effect(P>0.05) with other group.

Table 3: Composition of finisher diets(6-8Week)

Item	Control	5%Canola	10%Canola	15%Canola	20%Canola
Corn	70.81	67.64	65.6	63.56	65.89
Soybean meal	21.42	16	13.28	10.56	7.86
Fish meal	3	3	3	3	3
Canola meal	0	5	10	15	20
Fat	1.46	2.58	3.12	3.66	4.2
DCP	0.42	0.45	0.43	0.4	0.4
Oyster shell	1.18	1.06	1.02	0.98	0.95
Mineral premix	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.02	0	0	0	0
Salt	0.2	0.2	0.2	0.2	0.2
Calculated Composition					
ME (kcal/kg)	3084.9	3084.9	3084.9	3084.9	3084.9
CP(%)	17.35	17.35	17.35	17.35	17.35
Ca(%)	0.77	0.77	0.72	0.77	0.77
Total P(%)	0.28	0.29	0.29	0.29	0.29
Met+ Sys(%)	0.59	0.68	0.68	0.77	0.77
Lysine (%)	0.89	0.86	0.85	0.84	0.84

Table 4: Effect of dietary treatments on body weight gain and feed Conversion ratio

Item	Control	5%Canola	10%Canola	15%Canola	20%Canola
Body weight gain (g)					
0-3Week	27.36^{a}	37.02^{a}	27.23^{a}	26.9^{a}	24.2^{b}
3-6Week	53.43^{a}	50.02^{b}	54.5^{a}	53.03^{ab}	48.02^{b}
6-8Week	80.05^{a}	85.04^{b}	83.01^{ab}	78.01^{a}	72.05^{c}
0-8Week	52.1^{a}	56.01^{a}	53.2^{a}	44.02^{b}	42.05^{b}
Feed conversion ratio					
0-3Week	1.93	1.94	2.04	2.08	2.12
3-6Week	2.04^{a}	2.01^{a}	2.3^{b}	2.19^{b}	2.23^{b}
6-8Week	2.51^{a}	2.38^{a}	2.55^{a}	2.7^{b}	2.85^{b}
0-8Week	2.07^{a}	2.05^{a}	2.12^{a}	2.32^{b}	2.45^{b}

Table5:Effect of dietary treatments on carcass yield

Item	Control	5%Canola	10%Canola	15%Canola	20%Canola
Body weight (g)	2320.12^{b}	2450^{a}	2340.81^{b}	2112.01^{c}	2001.56^{c}
Carcass (%)	76.83	78.02	76.81	74.22	73.91
Abdominal fat (%)	2.13^{a}	2.28^{a}	2.24^{a}	2.75^{b}	2.81^{b}
Liver & gall bladder (%)	2.57	2.45	2.47	2.36	2.51
Intestinal (%)	4.46	4.36	4.32	4.51	4.6
Pancreas (%)	2.07^{a}	2.05^{a}	2.03^{a}	2.11^{a}	2.61^{b}

References

Ackman, R.G., 1990. Canola fatty acids-An ideal mixturefor health, nutrition, and food use. In: F. Shahidi(Ed.) Canola and Rapeseed, Production, Chemistry,Nutrition and Processing Technology. p: 81. VanNostrand Reinhold, New York.

Apata, D.F and V. Ojo, 2000. Efficacy of TrichodermaViride enzyme complex in broiler starters fedcowpea testa-based diets. In Animal Production in New millennium. Challenges and options. Proc. Of 25th NSAP Animal Conference, Michael OkparaUniversity of Agriculture, Umudike, p: 132-134.

Atteh, J.O. and F.D. Ologbenla, 1993. Replacement of Fish meal with maggots in broiler diets. Effects on performance and nutrient retention. Nig. J. Anim. Prod., 20: 44-49.

Bell, J. M., 1984. Starters fed cowpea testa -based diets. In Animal Production in New illennium. Challenges and options. Proc. of 25 NSAP Animal Conference, th Michael Okpara University of Agriculture, Umudike, p: 132-134.
Banerjee, G.C., 1992. Poultry, 3 edn. Oxford and IBH pub. Co.Pvt. Ltd. New Dilhi,Bombay, Calcata. Clark, W.D., H.L. Classen and R.W. Newkirk, 2001.Assessment of tail-end dhulled canola meal for use in broilers diets. Can. J. Anim. Sci., 81: 379-386.
Elwinger, K., 1986. Continued experiments with Newkirk, R.W. and H.L. Classen, 2002.The Effects of rapeseed meal of a Swedish low glucosinolate type fed to poultry. 2.An experiment with laying hens. Swed. J. Agri. Res., 16: 35-41.Economic Research Service (ERS), 2001. Oil crops situation and outlook. OCS-2000, Oct. 2001. ERS,USDA, p. 66.
Hill, R., 1979. A review of the toxic effects of rapeseed meal with observation on meal from improved varieties. Br. Vet. J., 135: 3-16. Igwebuike, J.U., I.D. Kwari, C.O. Ubosi and N.K. Alade, 2001. Replacement value of spent sorghum grains for maize in broiler finisher diets. J. Sustain. Agri. Environ., 3: 224-233.
Lee, P.A., S. Pittam and R. Hull, 1984. The volutary food intake by growing pigs of diets containing treated rapeseed meals of extracts of rapeseed meals. Br. J. Nutr., 52: 159-164.
Leeson, S., J.D. Summers, 1997. Commercial Poultry Nutrition. Department of Animal and poultry Science, second edition,University of Guelph, Guelph, Ontario, Canada. ISBN: 964-91901-4-7.
Murphy, M., P. Uden, D.L. Palmquist and H. Wiktorsson, 1987. Rumen and total diet digestibility in lactating cows fed diets containing full-fat rapeseed. J. Dairy Sci., 70: 1572.
Mutzar, A.J.H.J.A. Likuski, and S.J. Slinger, 1978. Metabolisable energy content of tower and candle rapeseeds and rapeseed meals determined in two laboratories. J. Biol.Chem. 238: 235-237.
Mutzar, A.J. and S.J. Slinger, 1980. Apparent amino acid availabilityand apparent metabolisable energy values of Tower and Candel rapeseeds and rapeseed meal in two laboratories. Can. J. Anim. Sci., 58: 485-492.
Nassar, A.R. and G.H. Arscott, 1986. Canola meal for broilers and the effects of dietary supplement of iodinated casein on performance and thyroid status. Nutrition Report International. 34: 791-799.
Najib, H. and S.A. Al-Khateeb, 2004. The Effect of Incorporating Different Levels of Locally Produced Canola Seeds (Brassica napus, L.) In the Diet of Laying Hen. Int. J. Poult. Sci., 3: 490-496.
Nworgu, F.C, E.A. Adebowale, O.A. Oredein and A. Oni, 1999. Prospects and economics of broiler production using two plant protein sources. Trop. J. Anim. Sci., 2: 159-166.
Ojewola, G.S., A.S. Eburuaja, F.C. Okoye, A.S. Lawal and A.H. Akinmutimi, 2003. Effect of inclusion of grasshopper meal on performance, Nutrient utilization and organ of Broiler chicken J. Sustain Agri. Environ., 5: 19-25.
Raymer, P.L., D.L. Auld and K.A. Mahler, 1990. Agronomy of canola in the United States. p. 25–35. In: F. Shahidi (ed.), Canola and rapeseed: Production, chemistry, nutrition, and processing technology. Van Nostrand Rhienhold, New York.
Roth-Maier, A. Dora and M. Kirchgessner, 1988. Feeding of 00-rapeseed to fattening chicken and laying hens. Landwirtsch. Forschung, 41:140-150.
Salmon, R.E., 1984. True metabolisable energy and dry matter contents of some feedstuffs. Poultry Sci., 63: 381-383.
Sibbald, I.R. 1977. The true metabolisable energy values of some feedstuffs. Poult. Sci. 56: 380-382.
Sibbald, I.R. and K. Price, 1977a. True metabolisable energy values of the seeds of Brassica campesyris, B. birta and B. napus.Poultry Sci.56:1329-133.
Sibbald, I.R., 1977b. The true metabolisable energy values of some feedstuffs. Poult. Sci., 56: 380-382.Singh, R.A., 1990. Poultry Production, 3rd edition.
Kalyany. Publishers, New Delhi, Ludhiana. Sosulski, F., 1974. Rapeseed Assoc.. of Canada 35 : 168.
Summers, J.D., S. Leeson and D. Spratt, 1988. Canola meal and egg size. Can. J. Anim. Sci., 68: 907-913.
Summers, J.D., S. Leeson, 1977. Effect of thyroxin and thiouracil addition to the diets containing rapeseed meal on chick growth and carcass composition. Poult. Sci., 56: 25-35.
Zeb, A., A. Satter and U. Meulen, 1999. Effect of feeding different levels of rapeseed meal on the performance of broiler chickens. Archive fuer Gefluegelkund, 63:77.

Meal quality improvement in *Brassica napus* canola through the development of low fibre (yellow-seeded) germplasm

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Abstract

The importance of the yellow seed trait in improving the feed value of canola meal through fibre reductions has been reported in various *Brassica* species by several researchers over a number of years. A strong correlation has been reported between seed colour and meal fibre reduction, in particular reduction in lignin content. Research at AAFC Saskatoon has utilized various sources of "yellow seed colour genes" in related *Brassica* species, and these were incorporated into adapted spring annual lines and cultivars through interspecific crosses. Interspecific F_1 plants were repeatedly backcrossed to *B. napus* canola followed by reselection of true breeding yellow-seeded phenotypes after each backcross for several inbred generations before the next backcross was carried out. The resulting yellow-seeded lines were field tested under Canadian prairie growing conditions for several years. In all cases, fibre contents were strongly correlated to seed colour with lowest fibre contents observed in lines with strongest expression of the yellow seed trait. Intensive breeding work will be required to develop agronomically superior yellow-seeded (low fibre) canola cultivars and hybrids.

Key words: Brassica napus, yellow seed, low fibre meal.

Introduction

The rapeseed (canola) oilseed crop in Canada is the summer annual form of *Brassica napus* with a low erucic acid content (<1% of total fatty acids) and a low glucosinolate content (<12 μmoles/g seed at 8% moisture). The seed oil must have a total saturated fat content of <7% of total fatty acids and the seed should have a low chlorophyll content of less than 25 mg/kg seed at maturity to qualify for the highest commercial grade, Canada No. 1, the basis for payment to the producer.

A high seed oil content is the most important seed quality objective in canola breeding because oil is the most important component of canola seed representing about 80% of its economic value. For this reason, minimum standards for oil content have been implemented for cultivar registration, together with minimum standards for meal protein contents. The average oil content of the Canadian canola crop as 42.8% at 8.5% seed moisture for the 10 year period 1995-2004 based on survey data by the Canadian Grain Commission (DeClerq, 2004). The average oil-free meal protein content, on an 8.5% moisture basis, for the same period was 40.4%. Oil and protein contents of individual farm fields (productions) can vary greatly due to environmental factors that affect plant growth and maturity.

Canola meal is viewed by the industry as a by-product of canola oilseed production and is used as an animal feed protein supplement. Canola meal has a high crude fibre content of about 12% compared to soybean meal containing about 4%, primarily a result of its small seed size. The meal is traded at a 35% price discount compared to soybean meal despite its good balance of amino acids (Bell, 1995). About 30% of the meal weight consists of seed coat tissue (hulls) which contains much less protein and more fibre than the seed embryo tissue. Hulls contain over 10% of total protein and 25% of gross energy of canola meal, and hulls in the meal depress levels of available energy and protein, as well as availability of amino acids and minerals (Bell, 1995). Therefore, a reduction in the hull content would be of interest to improve the nutritional value of the meal.

One possible and very efficient strategy to reduce the hull content in canola meal is the development of yellow-seeded cultivars which have been shown to have thinner seed coats than black-seeded cultivars, and meals produced from yellow seeds would therefore contain less hulls and a lower fibre content. This has clearly been shown in *B. rapa* (Stringam, et al. 1974), *B. juncea* (Woods, 1980) and *B. carinata* (Getinet et al. 1996) where yellow-seeded types do exist. Another advantage of the yellow seed trait is an increased oil content due to a larger embryo in yellow seed. *Brassica napus* cultivars and germplasm are black seeded and no yellow-seeded forms have ever been found in natural populations. Yellow-seeded *B. napus* has been developed through interspecific crosses with the objective to transfer the yellow seed trait from *B. rapa* into *B. napus* (Liu, 1983; Rahman, 2001), through resynthesis of *B. napus* based on crosses between yellow-seeded *B. rapa* and light seeded *B. alboglabra* (Shirzadegan, 1986; Chen et al. 1988) and through induced mutations (Sobrino-Vesperinas et al. 1991). More recently, yellow-seeded *B. napus* has also been successfully developed through interspecific crosses involving yellow-seeded forms of *B. juncea* and *B. carinata* in interspecific crosses with *B. napus* (Rashid et al. 1994).

This paper will review breeding work conducted at the AAFC Saskatoon Research Centre in regard to developing true breeding yellow-seeded forms of *B. napus* through interspecific crosses and improvements made in agronomic performance and seed quality.

Materials and Methods

Creation of true yellow breeding Brassica napus

Interspecific crosses were made at the University of Manitoba, Canada, (Dr. B.R. Stefansson) between yellow-seeded *B. carinata* as female and the black-seeded *B. napus* canola cultivar Regent and the F_1 backcrossed to Regent followed by selection of yellow-seeded plants in backcross generations. Selected yellow-brown seeded plants were then crossed with *B. rapa* yellow sarson and the resulting F_1 again backcrossed to Regent. Segregating generations of this second interspecific cross were selected for four generations for yellow seeded plants by Dr. D. Woods of AAFC Saskatoon and a yellow-brown seeded line, YSN80-1623 selected in the field.

YSN80-1623 was crossed as female with a F_5 generation inbred yellow-brown seeded AAFC *B. napus* canola quality line SZN73-1493, selected from crosses involving the rapeseed cultivars Target and Golden, zero erucic acid cultivars Oro and Zephyr and two low glucosinolate germplasm lines, Bronowski and S68-2895. The F_5 line YN86-37, selected from this cross, had yellow seed colour and was of canola quality.

YN86-37 was crossed in 1986, with a yellow-brown seeded *B. napus* introduction, obtained from the Svalöf seed company, R3608, resulting from the cross *B. napus* x *B. juncea* (yellow). The F_5 inbred line YN90-1016, selected from this cross was true breeding for the yellow seed trait. Selected yellow-seeded F_3 plants of this cross were crossed with the yellow-brown seeded *B. alboglabra* line 89-5402, derived from interspecific crosses with yellow-seeded *B. carinata* followed by backcrosses to *B. carinata* and reselection of yellow seeded plants. The F_1 of this cross was backcrossed to the yellow-seeded *B. napus* parent, and a yellow-seeded BCF_2 plant Rsyn 2-11 selected and this plant was further inbred to the BCF_5 generation to improve and stabilize the yellow seed trait.

Rashid et al. (1994) made two interspecific crosses: *B. napus* (Westar) x *B. juncea* (yellow) and *B. napus* x *B. carinata* (yellow) and backcrossed the two F_1 generations to *B. napus* (Westar). Twenty BCF_2 plants of the *B. juncea* interspecific cross were crossed with 20 BCF_2 plants of the *B. carinata* cross, and 18 doubled interspecific cross populations grown in field plots for selection of yellow-seeded plants. These were pedigree selected for several generations and a true yellow breeding, canola quality line, YN9592, identified in the F_6 generation.

Improving agronomic performance and seed quality of yellow-seeded B. napus

The yellow-seeded line YN90-1016 was crossed with the Australian blackleg resistant cultivar Shiralee and F_1 plants of this cross used as male parents and crossed with the black-seeded high oil content, AAFC Saskatoon canola line N89-53, a F_4 line derived from the cross Midas x Westar. The 3-way cross was selected for high oil content in plant-row progenies in the field and yellow seeds selected from highest oil content lines. The F_6 line YN97-262 was selected as a true breeding yellow seeded line in a field nursery in 1997.

Four black-seeded *B. napus* cultivars Magnum, Dunkeld, Range and Ebony were crossed as females with YN97-262 to improve yield, blackleg resistance, and oil and protein content of yellow-seeded *B. napus*. The F_1 Dunkeld x YN97-262 was crossed with the F_1 Magnum x YN97-262 to combine blackleg resistance with yield, and the F_1 Range x YN97-262 was crossed with the F_1 Ebony x YN97-262 to combine blackleg resistance with oil content. Double cross (DC) F_1 plants were interpollinated and DC F_2 plants grown in the field, followed by F_3 progeny evaluation of yellow-seeded DCF2 plants in a breeding nursery, and F_4 early generation yield assessments in replicated yield tests. The F_5 line YN01-429 was high yielding and had a high seed oil content and true breeding for the yellow seed trait which was confirmed in a F_6 plant-row progeny test in 2002.

The three yellow-seeded *B. napus* lines YN90-1016, Rsyn 2-11 and YN9592 were field tested in 4 replicate yield tests at Saskatoon, Scott and Melfort, Sask. from 2002-2004.

Results

Table 1: Yield and seed quality of yellow-seeded *Brassica napus* lines YN90-1016, Rsyn 2-11 and YN9592 of different interspecific origin and the agronomically improved true yellow-seeded line YN01-429 in comparison to the black-seeded cultivars 46 A65/Q2 in 4 replicate yield tests at Saskatoon, Scott and Melfort, Canada (total of 6 station years) from 2002 to 2004.

	Yield		Oil[1]	Protein[2]	Colour[3]	ADL[4]	ADF[5]
Lines	kg/ha	% checks	%seed	% meal	(WIE)	% meal	%meal
YN90-1016	1270	67	43.3	48.0	-22.4	1.2	7.8
Rsyn 2-11	1190	63	42.6	48.3	-22.9	1.0	7.7
YN9592	1610	85	43.8	47.5	-28.7	1.0	7.4
YN01-429	2030	107	49.1	46.9	-28.5	1.3	8.2
46 A65/Q2	1890	100	45.8	49.0	-0.8	5.4	13.6
SED	380		2.2	2.7		0.7	1.0
Station years	6		6	5	6	4	4

1=oil by NMR, 2=protein by LECO combustion, 3=seed colour by Method E313, white index, American Society for Testing and Materials. 4=ADL, acid detergent lignin, 5=ADF, acid detergent fibre (cellulose + lignin).

The true yellow-seeded lines derived from multiple interspecific crosses with yellow seeded donor species *B. carinata*, *B. rapa* yellow sarson and *B. juncea* for yellow seed colour, YN90-1016 and Rsyn 2-11 yielded 33% and 37%, respectively less

seed than the average of the two black-seeded check cultivars 46 A65/Q2 (Table 1). Their oil and protein contents were also lower than those of the checks. YN9592 yielded 85% of checks and had also lower oil and protein contents. The seed colour scores of -22.4 to -28.7 represent a good yellow colour compared to -0.8 for the check cultivars. Acid detergent lignin contents of the three original yellow seeded lines ranged from 1.0% to 1.2% of meal dry weight which represents about an 80% reduction in ADL compared to the black seeded cultivars. Acid detergent fibre levels were also low in the yellow seeded lines and were reduced by more than 50%.

The agronomically improved yellow-seeded line YN01-429 yielded 7% more seed than check cultivars and had a 3.3% higher seed oil content associated with a lower meal protein content. ADL and ADF fibre levels were low and comparable to those of the original yellow-seeded lines in combination with a strong yellow seed phenotype. All lines were of canola quality.

Discussion

We have shown that true breeding yellow-seeded lines of *B. napus* canola can be developed from interspecific crosses with related yellow seeded species of *B. carinata*, *B. rapa* yellow sarson and *B. juncea*. The recessive conditions of seed coat pigmentation genes, yellow seed, in the A-genomes of *B. rapa* (Stringam, 1980) and *B. juncea* (Vera et al. 1979) were successfully introgressed into the A-genome of *B. napus*, while yellow seed colour genes from the C-genome of *B. carinata* (Getinet & Rakow, 1997) were also successfully utilized and most likely incorporated into the C-genome of *B. napus*. A systematic genetic study on seed colour inheritance in *B. napus* is currently conducted to determine the number of genes involved in seed coat pigmentation in *B. napus* and to investigate their mode of inheritance.

The low seed yield of first generation yellow-seeded lines from interspecific crosses is most likely the result of aneuploidy and irregularities in meiotic cell division in these lines which we have observed (data not shown). These meiotic disturbances often lead to poor embryo development and premature seed ripening and seed abortion which causes low seed yield and reductions in seed oil content as observed in our material. However, systematic backcrossing of early generation yellow-seeded lines to agronomically superior, high oil and protein content elite cultivars or breeding lines will improve yield and quality of yellow-seeded lines as demonstrated by the development and performance of YN01-429. This line even outyields standard cultivars and has a significantly higher seed oil content. The higher oil content in YN01-429 results from a larger embryo in a bigger seed. The increase oil content in elite yellow-seeded lines will be of particular interest to the oilseed crushing industry, improving their crushing margins. YN01-429 has normal meiotic division, forming 19 bivalents and is fully fertile producing well developed seed of superior size.

We have shown that meal fibre contents, ADL and ADF in yellow seed meals are significantly reduced by about 75% and 50%, respectively. This reduction in meal fibre content will significantly increase the feed value of canola meal, and this has been documented in some animal feeding studies. Economic studies suggest that when adapted, high yielding yellow-seeded *B. napus* cultivars will be available for commercial production, the use of black-seeded cultivars will discontinue and the whole canola production will be of the yellow seed type (Evrard 2004). The commercial production of yellow-seeded cultivars of *B. napus* canola in Canada will improve the economic competitiveness of canola meal as a high quality protein feed supplement in animal feed relative to soybean meal.

Conclusions

The development and commercial production of adapted, high yielding, disease resistant, high quality yellow-seeded *B. napus* cultivars and hybrids will establish a new standard for the rapeseed (canola) industry worldwide. We believe that low fibre, high oil (yellow-seeded) canola must be the next quality breeding goal for *B. napus* canola and all further future oil and meal quality improvements must be built on this technology platform for the benefit of the whole canola industry.

References

Bell J.M. (1995). Meal and by-product utilization in animal nutrition. Chapter 14 In: *Brassica* oilseeds, production and utilization. Ed. D. Kimber and D.I. McGregor CAB International, Wallingford, Oxon, OX 10 8 DE, UK, pages 301-337.

Chen B.Y., Heneen W.K., Jönsson R. (1988). Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breeding **101**, 52-59.

DeClerq D.R. (2004). Quality of western Canadian canola, Canadian Grain Commission, Winnipeg, Canada.

Evrard J. (2004). Very low glucosinolate and yellow-seeded rapeseed: two genetic ways of increasing uses in animal feeding-an economical prospect. GCIRC bulletin No. **21**, December 2004, 7 pages.

Getinet A., Rakow G. (1997). Repression of seed coat pigmentation in Ethiopian mustard. Canadian Journal of Plant Science **77**, 501-505.

Getinet A., Rakow G., Downey R.K. (1996). Agronomic performance and seed quality of Ethiopian mustard in Saskatchewan. Canadian Journal of Plant Science **76**, 387-392.

Liu H.L. (1983). Studies on the breeding of yellow seeded *Brassica napus* L. Proceedings 6th International Rapeseed Congress, Paris, France. Vol. **1**, 637-641.

Rahman M.H. (2001). Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breeding **120**, 463-472.

Rashid A., Rakow G., Downey R.K. (1994). Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breeding **112**, 127-134.

Shirzadegan M. (1986). Inheritance of seed coat colour in *Brassica napus* L. Plant Breeding **96**, 140-146.

Sobrino-Vesperinas E., Fernandez M.C., Monroy F. (1991). Characteristics of a yellow seed line belonging to *Brassica* napus L. Proceedings 8th International Rapeseed Congress, Saskatoon, Canada. Vol. **1**, 203-210.

Stringam G.R., McGregor D.I., Pawlowski H.S. (1974). Chemical and morphological characteristics associated with seed coat colour in rapeseed. Proceedings 4th International Rapeseed Congress, Giessen, Germany, 99-108.

Stringam G.R. (1980). Inheritance of seed colour in turnip rape. Canadian Journal of Plant Science **60**, 331-335.

Vera C.L., Woods D.L., Downey R.K. (1979). Inheritance of seed coat colour in *Brassica juncea*. Canadian Journal of Plant Science **59**, 635-637.

Woods D.L. (1980). Association of yellow seed coat colour with other characteristics in mustard (*Brassica juncea*). Eucarpia Cruciferae Newsletter **5**, 23-24.

Stronger toasted rapeseed meals contain less glucosinolates, however, the *in vitro* protein quality is changed

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The glucosinolate concentration of solvent extracted rapeseed meal (RSM) depends on the glucosinolate content of the used seed batch and on the processing, particularly the temperature and the residence time in the toaster. Higher temperatures and a longer residence time in the toaster inactivate glucosinolates. In the oilmills, the bi-layered decks of the dryers (toasters) carrying the steamed (desolventized) meal are heated up to 130 ° C, with 20 to 60 min residence time (Münch 2005).

In a study, RSM from 10 oilmills differed very strongly in its glucosinolate content (SCHUMANN, 2005). In the present investigation, a stronger toasting was hypothesized for the RSM with lower glucosinolate content with the consequence of a protein damage which should be evidenced.

In RSM with differences of the glucosinolate content the concentration of the lysine as reference amino acid should be determined and its availability as possible measures of the degree of protein damage. Beside the amino acid analysis the homoarginine (HA) method was used to determine the available lysine. It bases on the guanidizing of the free epsilon amino group of the lysine to HA by a thiourea compound. Heat damaged lysine with blocked epsilon amino groups can not be guanidized and therefore it is determined unchanged as lysine and not as HA.

The ruminant produces high quality protein with all essential amino acids by the rumen microbes which is digested in the stomach and small intestine liberating all amino acids including the lysine. However, the amount of microbial protein seems to be not sufficient to meet the protein requirement of highly yielding cows, i.e. more than 35 litre milk per day. There is the possibility of an increased portion of the feed protein escaping the microbial degradation in the rumen and being available as direct feed amino acid source in the stomach/duodenum. More heat could change the portion of protein which passes the rumen and therefore the duodenally utilizable protein (uCP) and rumen undegradable protein (UDP) should also be investigated (for explanation see GfE, 2001).

Methods

Two types of RSM from two oilmills, with 10 samples each, either with lower GSL content of 2.4 ± 0.8 mmol/kg dry matter (DM or with higher GSL content of 13.8 ± 2.8 mmol/kg DM, were analysed (Tab 1). The different glucosinolate content of the two rapeseed batches indicates the different processing conditions with stronger meal toasting in oil mill A versus a weaker toasting in oil mill J.

Table 1 Glucosinolate concentration of rapeseed and rapeseed meals in the oil mills A and J (Schumann, 2005)

Oil mills Samples (number)	A	J
	mmol/kg dry matter	
Rapeseed	14,6 ± 5,9 (141)	15,3 ± 3,9 (153)
Rapeseed meal [1)]	2,5 ± 1,9 (80)	14,4 ± 3,2 (41)
Whereof for the present investigation	2,4 ± 0,8 (10)	13,8 ± 2,8 (10)

1) the glucosinolate content was analysed with the HPLC method according to EU (1990)
2)

The crude protein was determined as N (multiplied by the factor 6.25) according to the Kjeldahl method; for the amino acid analysis an amino analyser (Biochrom 20 from Pharmacia) was used (Bassler and Buchholz 1986).The detection of the available lysine via the HA method was described by Mauron and Bujard (1964). The method of determination of the uCP and that of the UDP according to the modified Hohenheim gas test is given by Steingass et al., 2001.

The results are given as arithmetic mean and standard deviation. The means were compared by STUDENTs` t test.

Results and discussion

The RSM with the lower glucosinolate content contained significantly less lysine, by 9 %, compared to the RSM with the higher glucosinolate content, although crude protein content was similar. In case of standardizing at 100 g crude protein the difference between the lysine concentrations rise to more than 10 %. The availability of the lysine measured by the guanidation of lysine with p-methyl isothiourea to HA differed significantly by 4.5 percent units, i.e. 6 % relatively. A lower

content and lower availability of the lysine in the stronger toasted RSM (indicated by the lower glucosinolate content) resulted in 2.5 g /kg meal less available lysine, i.e. 12 % less available lysine on meal basis and 15 % on basis of 100 g crude protein compared to the weaker toasted RSM (indicated by the higher glucosinolate content).

Regarding the protein measures for the ruminant the stronger toasted RSM contained 11 % more uCP and 20 % more UDP than the weaker toasted RSM. In both cases the significant difference means less microbial degradation of rapeseed protein in the rumen. More of such heat protected feed protein could be available in the duodenum as direct feed amino acid source complementary to the microbes` protein, suggesting that duodenal amino acid availability is less reduced than ruminal protein degradation.

Table 2: Protein quality of rapeseed meals from two oilmills (10 samples per meal)

Toasting	mmol/kg dry	Stronger			Weaker		
Glucosinolates	matter, DM	2.4 ± 0.8			13.8 ± 2.8		
Crude protein, CP	g/kg DM	394	±	12	387	±	9
Lysine	g/kg DM	20.3	±	0.8 [b]	22.2	±	0.8 [a]
Lysine	g/100 g CP	5.15	±	0.13 [b]	5.74	±	0.16 [a]
Available lysine	%	75.1	±	3.2 [b]	79.6	±	2.0 [a]
Available lysine	g/kg DM	15.2	±	0.8 [b]	17.7	±	0.7 [a]
Available lysine	g/100 g CP	3.86	±	0.18 [b]	4.56	±	0.16 [a]
Utilizable protein, uCP	g/kg DM	331	±	16 [a]	298	±	6 [b]
Undegradable protein, UDP	g/kg DM	230	±	20 [a]	192	±	10 [b]

[a,b] Means of the same line with different superscripts are significantly different.

Conclusion

A strong toasting of RSM in the oil mill increases the utilizable protein for the ruminant, however, it lowers the available lysine and thus the protein quality with regard to pig and poultry. Thus, RSM with lower glucosinolate content due to processing should be preferred for the ruminant feeding, however, for pigs and poultry a supply of extra lysine is required in such a strongly heated meal. Aiming the lowering of glucosinolate content of rapeseed the strategy of the plant breeding still has to have priority over certain measures in the processing. Further investigations in ruminants are necessary to define the reliable degree of feed protein protection in the rumen with a possibly high availability in the stomach and duodenum, respectively.

References

(Bassler, R.; H. Buchholz (ed.): Die chemische Untersuchung von Futtermitteln, Methodenbuch Bd. II, Verband der Landwirtschaftlichen Untersuchungs- und Forschungsanstalten, VDLUFA Verlag Darmstadt, einschließlich 4. Ergänzungslieferung, 1997)

European Community (1990). Oilseeds - determination of glucosinolates - high performance liquid chromatography. *Official Journal of European Commission*. L 170, 27-34.

GfE - Gesellschaft für Ernährungsphysiologie der Haustiere (2001): Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlags GmbH Frankfurt am Main, 136 Seiten

Mauron, J.; E. Bujard (1964): Guanidination, an alternative approach to the determination of available lysine in food. Proc. 6th Int. Congr. Nutr. Edinburgh, UK, 489-490

Münch, E: LIPPRO Consulting Verden: Personal information, Mai 2005

Schumann, W. (2005): Untersuchungen zum Glucosinolatgehalt von in Deutschland erzeugten und verarbeiteten Rapssaaten und Rapsfuttermitteln. Union zur Förderung von Öl- und Proteinpflanzen e.. V. Berlin UFOP- Schriften, Heft 27, Seiten 40 und 44

Steingaß, H.; D. Nibbe, K.-H. Südekum, P. Lebzien, H. Spiekers (2001): Schätzung des nXP-Gehaltes mit Hilfe des modifizierten Hohenheimer Futterwerttests. Abstracts 113. VDLUFA-Kongress, Berlin, 114. * The investigation was sponsored by the German Oil Millers` Association (Verband Deutscher Ölmühlen e.V. Berlin).

Effects of enzyme addition on the nutritive value of broiler diets containing high proportions of hulled or dehulled Chinese double-low rapeseed meals

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Abstract

This study was conducted to investigate the effect of fibre-degrading enzymes on the nutritive value of broiler diets containing high proportions of hulled or dehulled Chinese double-low rapeseed meals (DLRM). Two two-phase basal diets (phase 1, 4 ~ 21 d of age; phase 2, 22 ~ 42 d of age) were formulated with either hulled (22.5% and 23.5% of diet for phase 1 and 2, respectively) or dehulled (20% and 21.5% of diet for phase 1 and 2, respectively) Chinese DLRM as the major protein source to meet NRC (1994) nutrient requirements. The two basal diets, respectively, plus enzymes A (xylanase + β-glucanase), B (xylanase) and C (xylanase + cellulase) created another six diets. The eight grower diets with triplicate each were used to predict responses of diets to exogenous enzymes by the in vitro two-stage incubation method. Subsequently, a two-phase performance trial was conducted with 288 four-d-old chickens assigned to eight diets with 6 replicate floor pens of 6 birds each. Overall, the digestibility of DM or NDF didn't differ ($P > 0.05$) due to meal types; enzymes B and C addition either to hulled or dehulled DLRM diets both resulted in increased ($P < 0.05$) CP and NDF digestibility compared with their respective controls. Birds fed dehulled DLRM diets had a higher ($P < 0.05$) growth rate, feed efficiency and lower ($P < 0.05$) feed intake than those feed hulled DLRM diets. Enzyme C addition to dehulled DLRM diets resulted in improved ($P < 0.05$) growth rate and feed efficiency during phase 1. Enzymes A and B addition elicited a positive response in feed intake and weight gain ($P < 0.05$), respectively. However, feed efficiency was affected ($P > 0.05$) by neither of the two enzymes. It would appear feasible that using appropriate fibre-degrading enzymes to improve feeding values of broiler diets containing Chinese DLRM. Responses of broilers to fibre-degrading enzymes could be highlighted by hull removal of fed DLRM.

Key words: Broilers, Chinese double-low rapeseed meal, digestibility, performance

Introduction

Double-low rapeseed meal (DLRM), more commonly known as canola meal is considered as a good protein source in poultry diets. Unfortunately, the relatively low digestible energy resulting from the high fibre content (Bell, 1993) still limits its dietary inclusion level. Mechanical dehulling was considered an alternative to reduce fibre content and, consequently, enhance the nutritive value. However, the reduction in dietary fibre following removal of hulls was mainly reflected by a decrease in insoluble fibre, lignin in particular, but total non-starch polysaccharides (NSP) still accounts for some 17.8-21.4%, as near as making no difference from that present in hulled DLRM (16-22%) (Campbell et al., 1995). At the same time, hull removal may cause an increased level of soluble fibre and worse viscosity problem for that a majority of soluble fibre is present in cotyledon of DLRM (Peng, 2001).In addition, analysis in our laboratory indicated that neutral detergent fibre (NDF) content of dehulled Chinese DLRM is 19.5% (as feed), approximately 50% higher than that of soybean meal (13.3%, NRC 1994), whereas NDF negatively affected energy and protein digestibility (Schullze et al., 1994). In short, the potentially increased soluble fibre and the remained high content of fibres such as NDF or NSP still limit the feeding value of DLRM although hull removal decreased fibre content in some degree.

Using fibre-degrading enzymes to breakdown NSP is considered to be a promising way that reducing the anti-nutritional effect of these fibre components in animal feeds. For DLRM, a total of about 15% carbohydrates including starch, free sugars and soluble NSP are encapsulated by cell walls and their actual contribution to digestible energy is modest (Bell, 1993). In this regard, adding an effective enzyme targeting cell wall fibres may improve the nutritive value of DLRM. In addition, the potential viscosity problem caused by relatively increased soluble fibre seems to make the enzyme addition to dehulled DLRM diet become more necessary (Tang et al., 2006).

In the present study, the first consideration was to comparatively evaluate responses of broilers to diets containing different types of DLRM (hulled versus dehulled). Another objective was to investigate the feasibility of using fibre-degrading enzymes to improve the nutritive value of broiler diets containing high proportions of hulled or dehulled Chinese DLRM as estimated from in vitro nutrient digestibility and broiler performance.

Material and methods

*Basal diets and treatments:*Two two-phase basal diets for broilers (phase 1, 4 to 21 d of age; phase 2, 22 to 42 d of age) were formulated with either hulled or dehulled Chinese DLRM as the major protein source to meet NRC (1994) nutrient requirements. Basal diet 1 contained 22.5% (phase 1) and 23.5% (phase 2) hulled Chinese DLRM, and basal diet 2 contained

20% (phase 1) and 21.5% (phase 2) dehulled Chinese DLRM. The two basal diets, respectively, plus enzymes A (xylanase + β-glucanase), B (xylanase) and C (xylanase + cellulase) created another six diets. Three enzymes were all in powder form, and were directly added to the complete diet. All diets were in mash form. The diet formulations were presented in Table 1.

Table 1. Basal diet formulation (as fed basis)

Ingredients (g/kg)	Grower phase (4 ~ 21 d)		Finisher phase (22 ~ 42 d)	
	Basal diet 1	Basal diet 2	Basal diet 1	Basal diet 2
Corn	517.4	562.3	564	609
Soybean meal	190	185	125	120
Hulled Chinese double-low rapeseed meal	225	0	235	0
Dehulled Chinese double-low rapeseed meal	0	200	0	215
Methionine	1.3	1.4	1.2	1.3
Lysine	2.5	1.5	2.8	1.6
Salt	3.8	3.8	3.6	3.6
Soybean oil	25	10	35	15
Limestone	10	12	9	11
Dicalcium phosphate	20.0	19.0	19.4	18.5
Premix*	5	5	5	5
Nutrients as calculation				
ME (MJ/kg)	121.3	121.3	125.5	125.5
Crude protein	210	210	190	190
Salt	3.7	3.7	3.5	3.5
Calcium	10	10	9.5	9.5
Total phosphorus	7.8	7.8	7.5	7.5
Available phosphorus	4.7	4.7	4.5	4.5
Digestible lysine	10	10	8.9	8.9
Digestible Methionine+Cystine	7.2	7.2	6.7	6.7

*Provided per kg of diet: Vitamin A, 13500 IU; Vitamin D3, 3000 IU; Vitamin E, 22.5 mg; menadione, 3.0 mg; thiamine, 3.0 mg; riboflavin, 7.5 mg; niacin, 30 mg; d-panthothenic acid, 15 mg; Vitamin B6, 3.0 mg; Vitamin B12, 23 μg; d-biotin, 120 μg; folic acid, 1.5 mg. copper, 11 mg; iron, 100 mg; manganese, 110 mg; zinc, 100 mg; iodine, 0.8 mg; selenium, 0.3 mg. Arsanilic Acid, 90mg; Zinc Bacitracin, 50 mg.

In vitro two-stage enzyme incubation trial: The eight diets for phase 1 were incubated in triplicate with the *in vitro* two-stage enzyme incubation and dialysis procedure as described in detail by Peng (2000). The residues from the dialysis tubes were then frozen, freeze-dried and analyzed for DM and CP using the technique outlined by AOAC (1990). NDF content in diets and residue was determined by the method of Goering and Van Soest (1970). Each sample was analyzed in duplicate and the *in vitro* digestible DM, CP and NDF were calculated by subtracting the amount of DM, CP and NDF remaining in the residue from the present in the original diet. The digestibility coefficients were calculated from the following equation (taken CP as an example): CP digestibility coefficient = digestible CP (g/kg diet)/total dietary CP (g/kg diet)

Table 2. Effects of enzyme addition on in vitro digestibility of DM, CP and NDF. Values are means ± SD (n = 3)

Meal	Enzyme	DM (%)	CP (%)	NDF (%)
Hulled meal	Control	42.82 ± 1.90^{c}	53.50 ± 0.09^{e}	11.11 ± 1.34^{e}
Hulled meal	Enzyme A	43.81 ± 0.16^{bc}	56.38 ± 1.00^{d}	16.21 ± 0.72^{bcd}
Hulled meal	Enzyme B	45.34 ± 0.43^{ab}	58.70 ± 0.27^{ab}	20.12 ± 2.40^{b}
Hulled meal	Enzyme C	45.86 ± 0.94^{ab}	56.63 ± 1.22^{cd}	25.39 ± 2.14^{a}
Dehulled meal	Control	44.01 ± 2.14^{bc}	57.16 ± 1.02^{bcd}	13.63 ± 1.91^{de}
Dehulled meal	Enzyme A	45.84 ± 1.19^{ab}	58.45 ± 0.91^{abc}	14.67 ± 1.80^{cde}
Dehulled meal	Enzyme B	44.63 ± 0.71^{abc}	60.38 ± 0.12^{a}	19.33 ± 2.81^{bc}
Dehulled meal	Enzyme C	46.69 ± 0.87^{a}	59.64 ± 1.06^{a}	19.54 ± 2.20^{bc}
			—probability of greater *F* value in ANOVA—	
Source of variance				
Meals		NS	***	NS
Enzyme		*	***	***
Meal × Enzyme		NS	NS	0.10

[a-c]Values within a column with no common superscripts differ significantly ($P < 0.05$)
[***]$P < 0.001$, [*] $P < 0.05$

Feeding Trial: Single sex (male) Avian broiler chickens were raised from hatch to 4 d of age in brooders on commercial starter crumbles. At d 4, a total of 288 four-d-old chickens were used in the performance trial, and chickens were randomized among 48 floor pens with each 2 m^2 pen containing 6 chickens, which means six replicates (pens) per dietary treatment. Clean wood shavings were used as litter. Chickens had free access to feed and water. Lightening program, temperature and relative

humidity were according to conventional conditions. Temperature and relative humidity were recorded daily. Feed intake per pen was recorded daily throughout the experiment, and body weight data was recorded at d 21 and 42 of age. The total experiment conducted in two phases (growing phase, 4 ~ 21 d of age; finishing phase, 22 ~ 42 d of age) lasted 39 d.

Statistical Analysis: The study was conducted in a randomized complete block design. SAS (1989) was used to perform the statistical analysis used in this study. Data were analyzed according to the GLM procedure for ANOVA to determine the significance of the main effects (DLRM and enzyme addition) and interactions with the mean value of a pen as the experimental unit, and Duncan's multiple range test was used to separate means when significant effects ($P < 0.05$) were detected by multifactorial analysis of variance.

Results and discussion

Effects of meal type (hulled vs. dehulled) and enzyme addition on the in vitro digestibility

The in vitro digestibility of DM, CP and NDF following enzyme supplementation was shown in Table 2. Overall, the digestibility of DM or NDF was not significantly ($P > 0.05$) different due to meal types, whereas dehulled DLRM diets had a significantly ($P < 0.001$) higher CP digestibility than hulled DLRM diets, regardless of enzyme addition or not. Among the three enzymes, enzymes B and C addition either to hulled or dehulled DLRM diets both resulted in significantly ($P < 0.05$) increased CP and NDF digestibility compared with their respective controls. In contrast, a significant enhancement in CP and NDF digestibility was observed in hulled DLRM diets rather than in dehulled DLRM diets following enzyme A supplementation. Remarkably, with the inclusion of enzyme C, NDF digestibility was improved by 1.3-fold in hulled DLRM diet compared with 0.4-fold in dehulled DLRM diet. This could be explained by the difference in fibre components between hulled and dehulled DLRM. It was reported that the major NSP components found in DLRM were pectic polysaccharides, which include rhamnogalacturonan with associated side chains consisting of arabinose, galactose, and xylanase residues (Bacic et al., 1988). Further study revealed that the non-cellulase polysaccharides in DLRM consisted of arabinose (33%), xylose (13%), mannose (3%), rhamnose (2%), fucose (2%), uronic acids (30%), galactose (13%) and glucose (5%) (Slominski and Campbell, 1990). The high content of arabinose and xylose in DLRM indicated the presence of considerable amount of arabinoxylans (Slominski and Campbell, 1990), which with other polysaccharides including cellulase, xylans, and xyloglucans, are predominantly found in the hull fraction (Meng and Slominski, 2005). In this regard, more amounts of substrates for fibre-degrading enzymes such as cellulase and xylanase would be available in hulled DLRM diet than in dehulled DLRM, which may highlight the responses of DLRM-containing diets to fibre-degrading enzymes in terms of fibre degradation and, consequently, a higher improvement of NDF digestibility was observed for hulled DLRM diets compared with that for dehulled DLRM diets following the same enzyme addition. The varied NDF digestibility of hulled DLRM diets with the inclusion of different enzymes may be a result of the difference in enzyme components and sources. Xylanase, for example, even derived from the same source organism, can vary widely in their catalytic activities on various xylan substrates (Bedford and Schulze, 1998; Faulds et al., 2003; Frederix et al., 2003).

Table 3. Feed intake of broilers fed Chinese double-low rapeseed meal diets with and without enzyme addition. Values are means ± SD (n = 6)

Meal	Enzyme	Feed intake (g/d)		
		Grower phase (4 ~ 21 d)	Finisher phase (22 ~ 42 d)	Overall phase (4 ~ 42 d)
Hulled meal	Control	43.5 ± 1.0[a]	131.8 ± 5.0[a]	91.1 ± 2.9[a]
Hulled meal	Enzyme A	42.7 ± 1.3[ab]	127.6 ± 4.7[ab]	88.4 ± 3.0[ab]
Hulled meal	Enzyme B	43.4 ± 2.0[a]	131.8 ± 6.1[a]	91.0 ± 3.9[a]
Hulled meal	Enzyme C	43.4 ± 0.7[a]	132.3 ± 6.1[a]	91.3 ± 3.1[a]
Dehulled meal	Control	42.3 ± 1.4[ab]	122.6 ± 6.6[b]	85.5 ± 3.9[b]
Dehulled meal	Enzyme A	43.2 ± 1.3[ab]	130.0 ± 3.4[a]	90.0 ± 1.9[a]
Dehulled meal	Enzyme B	43.3 ± 0.6[a]	126.8 ± 6.6[ab]	88.3 ± 3.7[ab]
Dehulled meal	Enzyme C	41.8 ± 1.6[b]	127.9 ± 4.8[ab]	88.2 ± 3.3[ab]
		—probability of greater *F* value in ANOVA—		
Source of variance				
Meals		NS	*	*
Enzyme		NS	NS	NS
Meal × Enzyme		NS	0.09	0.08

[a-b]Values within a column with no common superscripts differ significantly ($P < 0.05$)
*$P < 0.05$

Effects of Meal Type and enzyme addition on Broiler Performance

To comparatively evaluate responses of broilers to diets containing different types of DLRM (hulled versus dehulled), we incorporated the two basal diets on calculated equal-energy (ME) and equal-protein basis with hulled or dehulled DLRM as the major protein source. Feed intake, growth rates and feed conversion ratio measured over the grower phase (4 ~ 21 d), finisher phase (22 ~ 42 d) and overall phase (4 ~ 42 d) were shown in Tables 3, 4 and 5, respectively. Interestingly, no difference in feed intake was observed between the two DLRM diets during the growing phase. In contrast, during the finishing and overall phase birds fed dehulled DLRM diets had a significantly lower feed intake than those fed hulled DLRM

diets. Consequently, during these two phases, birds fed dehulled DLRM diets had a significantly higher feed efficiency (gain:feed) than those fed hulled DLRM diets. Considering that dietary available energy was normally the determinant factor that affects the feed intake of birds, the relatively lower feed consumption for dehulled DLRM diets may suggest that the available energy of dehulled DLRM be underestimated when the diets were formulated.

Table 4. Average daily gain of broilers fed Chinese double-low rapeseed meal diets with and without enzyme addition. Values are means ± SD (n = 6)

Meal	Enzyme	Average daily gain (g/d)		
		Grower phase (4 ~ 21 d)	Finisher phase (22 ~ 42 d)	Overall phase (4 ~ 42 d)
Hulled meal	Control	33.4 ± 0.8^{b}	64.7 ± 4.3^{ab}	50.2 ± 2.5^{abc}
Hulled meal	Enzyme A	31.7 ± 1.1^{c}	62.7 ± 2.7^{b}	48.4 ± 1.8^{c}
Hulled meal	Enzyme B	33.6 ± 1.2^{b}	65.3 ± 2.5^{ab}	50.7 ± 1.1^{abc}
Hulled meal	Enzyme C	34.0 ± 1.2^{ab}	63.8 ± 4.8^{ab}	50.1 ± 2.7^{bc}
Dehulled meal	Control	33.3 ± 2.5^{b}	65.4 ± 4.8^{ab}	50.6 ± 2.5^{abc}
Dehulled meal	Enzyme A	34.8 ± 1.6^{ab}	66.9 ± 2.6^{a}	52.1 ± 1.3^{ab}
Dehulled meal	Enzyme B	35.7 ± 0.6^{a}	61.7 ± 2.4^{b}	49.7 ± 1.6^{c}
Dehulled meal	Enzyme C	35.4 ± 1.2^{a}	67.1 ± 3.3^{a}	52.5 ± 1.9^{a}
		—probability of greater *F* value in ANOVA—		
Source of variance				
Meals		***	NS	*
Enzyme		**	NS	NS
Meal × Enzyme		*	*	*

[a-c]Values within a column with no common superscripts differ significantly (P < 0.05)
[***]P < 0.001, [**]P < 0.01, [*]P < 0.05

Table 5. Feed:gain of broilers fed Chinese double-low rapeseed meal diets with and without enzyme addition. Values are means ± SD (n = 6)

Meal	Enzyme	Feed:gain ratio		
		Grower phase (4 ~ 21 d)	Finisher phase (22 ~ 42 d)	Overall phase (4 ~ 42 d)
Hulled meal	Control	1.31 ± 0.04^{ab}	2.05 ± 0.18^{ab}	1.82 ± 0.12^{a}
Hulled meal	Enzyme A	1.35 ± 0.03^{a}	2.04 ± 0.07^{ab}	1.83 ± 0.06^{a}
Hulled meal	Enzyme B	1.29 ± 0.05^{ab}	2.02 ± 0.10^{abc}	1.80 ± 0.07^{a}
Hulled meal	Enzyme C	1.28 ± 0.05^{b}	2.08 ± 0.13^{a}	1.83 ± 0.09^{a}
Dehulled meal	Control	1.27 ± 0.09^{bc}	1.88 ± 0.20^{c}	1.69 ± 0.13^{b}
Dehulled meal	Enzyme A	1.24 ± 0.06^{bc}	1.94 ± 0.07^{abc}	1.73 ± 0.05^{ab}
Dehulled meal	Enzyme B	1.22 ± 0.03^{cd}	2.06 ± 0.14^{a}	1.78 ± 0.09^{ab}
Dehulled meal	Enzyme C	1.18 ± 0.05^{d}	1.91 ± 0.06^{bc}	1.68 ± 0.04^{b}
		—probability of greater *F* value in ANOVA—		
Source of variance				
Meals		***	*	***
Enzyme		*	NS	NS
Meal × Enzyme		NS	NS	NS

[a-d]Values within a column with no common superscripts differ significantly (P < 0.05)
[***]P < 0.001; [**]P < 0.01; [*]P < 0.05

As for the growth rate, the results of two-way ANOVA analysis showed that birds fed dehulled DLRM had a higher (P < 0.05) growth rate than those fed hulled DLRM during the grower and overall phase, respectively. This seemed contradictory to Campbell et al. (1995). However, it needs to note that a significant interaction between enzymes and meal types occurred during these two phases. In detail, enzyme supplement in dehulled DLRM diets resulted in significantly improved weight gain, but not in hulled DLRM diets. Furthermore, during any experimental phase, there were no differences in growth rate between the two controls containing hulled and dehulled DLRM, respectively. Therefore, it would appear that the observed difference was resulted from enzyme addition, not from the change in meal type. In contrast, Kracht et al. (1999) compared the influence of graded rapeseed meal levels (7%, 14%, 21%) from hulled and dehulled rapeseed on growth performance and found that the in the average of the three levels the weight gain of broilers fed dehulled rapeseed meal diets rose about 53 g (=3.5%) compared with that fed hulled rapeseed meal diets although at a substitution level of 21% the growth decreased. The inconsistency may be associated with the difference in diet formulation between these studies. Remarkably, to show the effect of dehulling, the energy content of the diets was not equalized in the study by Kracht et al. (1999), whereas soybean oil were used as an energy supplement to achieve equalized energy for the two types of DLRM diets in the present study. Therefore, the equalized energy may in part mask and, consequently, result in underestimate of the improved feeding values of dehulled DLRM in comparison to hulled DLRM.

Overall, enzyme C had a higher efficacy than enzymes A and B. For example, enzyme C addition to birds aged 4 to 21 d increased weight gain by 5.9% (35.4 g/d vs. 33.3 g/d, $P < 0.05$), and decreased feed:gain ratio by 7.1% (1.18 vs. 1.27, $P < 0.05$) compared with the control without enzyme addition. Also, birds fed enzyme C-supplemented diet had the highest feed efficiency over the total experimental phase. In contrast, adding enzyme B to dehulled DLRM diets enhanced the growth rate of birds aged 4 to 21 d, but the feed efficiency was not improved. Similarly, enzyme A had a positive effect on feed intake, but its adding value was discounted by the resulted high feed:gain ratio. Remarkably, in the current study, improved growth performance by enzyme supplementation was observed in dehulled DLRM diets but not in hulled DLRM diets, although enzyme addition to either of the two types of diets both resulted in increased nutrient digestibility in vitro. As discussed previously, the difference in responses of the two types of diets to similar enzyme addition may be associated with the difference in fibre components and their anti-nutritional effects. Furthermore, water-soluble NSP seemed to be more susceptible to enzyme action especially under a short digesta transit time in the gastrointestinal tract (Danicke et al., 1999; Meng and Slominski, 2005). It would appear that dehulled DLRM may produce more complex anti-nutritional effect than hulled DLRM when incorporated into broiler diets and, consequently, hull removal of DLRM may highlight the responses of broilers fed DLRM diets to exogenous enzymes as evidenced from the current study.

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References

AOAC. (1990). Association of Official Analytical Chemists. Official Methods of Analysis; S. Williams, ed. AOAC: Arlington, VA.

Bacic A., Harris P.J., Stone B.A. (1988). Structure and function of plant cell walls. Pages 297-372 in The Biochemistry of Plants. Vol 14. J. Preiss, ed. Academic Press, Inc., London.

Bedford M. R., Schulz H. (1998). Exogenous enzymes in pigs and poultry. Nutrition Research Reviews 11, 91-114.

Bell J. M. 1993. Factors affecting the nutritional value of canola meals: A review. Canadian Journal of Animal Science **73**, 679-697.

Campbell L.D., Simbaya J., Zhang W., Slominski B.A., Guenter W. (1995). Nutritive value of dehulled canola meal. Pages 179-181 in Proceedings of the 9th International Rapeseed Congress. Cambridge, UK.

Danicke S., Dusel G., Jeroch H., Kluge H. (1999). Factors affecting efficiency of NSP-degrading enzymes in rations for pigs and poultry. Agribiology Research **52**, 1-24.

Faulds C.B., Zanichelli D., Crepin V.F., Connerton I.F., Juge N., Bhat M.K., Waldron K.W. (2003). Specificity of feruloyl esterases for water-extractable and water-unextractable feruloylated polysaccharides: influence of xylanase. Journal of Cereal Science **38**, 281-288.

Frederix S.A., Courtin C., Delcour J.A. (2003). Impact of xylanases with different substrate selectivity on gluten–starch separation of wheat flour. Journal of Agricultural and Food Chemistry **51**, 7338–7345.

Kracht W., Jeroch H., Daenicke S., Matzke W. (1999). Effect of dehulling rapeseed on feed value of rapeseed meal and cake for poultry. In Proceedings of the 10th international rapeseed congress, Canberra, Australia.

Meng X., Slominski B.A. (2005). Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a muticarbohydrase preparation of cell wall degrading enzymes. Poultry Science. **84**:1242-1251.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Peng J. (2000). Evaluation and Improvement of Quality of Chinese Canola Meal. Ph.D. Diss., Huazhong Agric. Univ., Wuhan, Hubei.

Peng J., Slominski B.A., Guenter W., Campbell L.D., Xiong, Y.Z. (2001). The antinutritional factors in Chinese canola meal. Journal of the Chinese Cereals and Oils Association **16** (5), 6-9.

Schulze H., van Leeuwen P., Verstegen M.W., Huisman J., Souffrant W.B., Ahrens F. (1994). Effect of level of dietary neutral detergent fiber on ileal apparent digestibility and ileal nitrogen losses in pigs. Journal of Animal Science **72**, 2362-2368.

SAS Institute Inc. 1989. SAS/STAT User's Guide: Version 6. 4th edn. SAS Institute Inc., Cary, North Carolina.

Slominski B.A., Campbell L.D. (1990). Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. Journal of the Science of Food and Agriculture **53**, 175-184.

Tang T.J. (2006). Effects of enzyme additives in wheat- and double-low rapeseed meal-based deits on performance of growing-finishing pigs. Journal of Huazhong Agricultural. University **25**, 159-163.

Development of technology for detoxification of Indian mustard deoiled cake for poultry and livestock consumption

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Abstract

India produces almost 42 lakh tones of mustard annually with protein content of 37-38%. Mustard de-oiled cake (DOC) is the best and cheap source of protein for livestock and poultry in India, but is not being utilized properly as a direct feed for livestock due to the presence of antinutritional elements such as allylisothiocyanate, phenolic and phytic acid. The amount of allylisothiocyanate present in mustard DOC is 40-54 μmoles/gm and is sufficient to cause goiter in poultry. Phytic acid forms 2.3-2.5% of cake and phenolic make around 3.0-3.5% of it. Therefore, detoxification of mustard cake at this juncture is important for proper and efficient utilization of DOC as a livestock feed. But unfortunately, till date no such technology has been developed for its commercialization. In our research various experiments were undertaken to develop a technology for detoxification of mustard DOC. In first experiment, the DOC was treated with a solution of 10% NH_4OH and 1% ethanol at 80°C for half an hour followed by continuous washing with water. The quantification of various anti-nutrients was done following AOCS protocols. This detoxification process has shown promising results but cannot be used commercially as alcohol use will put huge pressure on industry's economic viability. In other experiment acetic acid treatments of DOC with simultaneous baking at 65°C was done for its successful detoxification where acetic acid (0.5% of DOC) treatment for 3 hours at 65°C with constant moisture level of 7% is sufficient to reduce the phytic acid up to as low as 1.91% and reduces the phenolic content up to 1.71%. In another experiment, the treatment of DOC with saturated steam at pressure of 15 psi found to be the most effective in its maximum detoxification, as the phytic acid concentration was reduced from 2.53% in original cake to 0.35% in cake after its treatment and phenolic concentration was reduced from 3.153% to 0.791%. The residual cake was found to be palatable for poultry, as it is free from the said antinutritional elements. The last experiment has the potential to be developed as technology for DOC detoxification and can be exploited for commercial use.

Key words: Detoxification, deoiled cake, allylisothiocyanate, phytic acid, phenolic, antinutritional elements, saturated steam.

Introduction

Mustard is used for production of high quality edible oil and a feed-grade meal. Mustard meal left after oil extraction from seeds is an excellent source of about 40% proteins, has reasonably well-balanced amino acid content (Ohlson, 1978) and favorable protein efficiency ratio (Delisle et al., 1984) is better than soy protein. Besides, so much of potential, utilization of mustard meal as a source of protein in livestock nutrition is limited due to the presence of allylisothiocyanate, phytic acid, and phenolic compounds. Presence of allylisothiocyanate in the diets leads to hyperthyroidism in animal and poultry, reduces the level of circulating thyroid hormones and alters the ratio between T3 and T4 in blood. Phenolic compounds contributed to the dark color, bitter taste and astringency of meals. They and/or their oxidized product also may form complex with essential amino acid enzymes and other proteins, thus lowering the nutritional value (Kumar et al., 1984). Phytic acid is a powerful chelator, especially of polyvalent cations, which are bound more strongly than monovalent cations (Graf, 1986) thereby markedly reducing the bioavailability of several multivalent cations by forming insoluble, phytate metal complexes. Moreover, the phytate also decrease protein digestibility through formation of indigestible protein-phytate complex. Therefore, much research has been directed towards detoxification of mustard meal from these antinutritional elements to make it palatable for livestock. The detoxification procedures investigated can be grouped into the following five categories: 1. Potential of the enzyme and removal of the hydrolytic products. 2. Inactivation of enzyme myrosinase. 3. Removal of antinutritional elements from mustard meal. 4. Destruction of antinutritional elements present in meal. 5. Breeding out antinutritional elements from crop. But till date not a single method has been developed which reduce all the antinutritional elements in a single treatment up to the extent which do not cause any detrimental effect to livestock. So, in this process we tried to develop a technology for detoxification of mustard deoiled cake without any further deterioration in the quality of meal.

Material and Methods

Phytic Acid Quantification: The phytic acid was extracted and determined according to the supernatant difference method of Thompson and Erdman (1982) with minor modifications. 1 gm sample was extracted with 25ml of 15% Trichloroacetic acid (TCA) containing 10% Sodium sulfate by mechanical shaking for 30 min followed by centrifugation at 12100x g for 15 min in REMI cooling Compufage CPR30. The supernatant was decanted and the pellet was re-extracted with another 25 ml for 30 min and again supernatant was decanted in previously obtained supernatant. The pellets were then collected together and phytic acid was precipitated with 1% $FeCl_3$ solution. The amount of phytic acid was obtained by the

difference of the phosphorus value between the initial supernatant and after precipitation with $FeCl_3$.

Allylisothiocyanate Quantification: The allylisothiocyanate were quantified according to Directorate General of Health Service Government of India protocol for allylisothiocyanate. In this process 5gm of mustard cake powder was taken in round bottom flask with 25 ml ethanol and 250 ml of glass distilled water and subjected to distillation using Liebig's condenser and the condensate was collected in a conical flask containing 25 ml 0.1 N $AgNO_3$ along with 10 ml 10% NH_4OH without any exposure to air till the solution in the flask made up to 150 ml. Along with it a blank was also placed. Then the solution was placed for aging using air column below 100^0C for 1 hr. After cooling make it to 200 ml by adding water. Out of this 100ml was taken and was titrated with 0.1N Ammonium thiocyanate till pink color develops. Volume of ammonium thiocyanate used was noted in sample as well as in blank.

Allylisothiocyanate content was quantified by using following formula:

$$\frac{9.915 \text{ X (Blank – Sample) X Normality of Solution used}}{\text{Weight of Sample}}$$

Phenolic Quantification: Phenolic in mustard meal was qualitatively tested using alcoholic $FeCl_3$ solution. About 2 g sample was extracted with ethanol (1: 6 w/v) for 1hr and the extracted volume was reduced in vacuum. The reduced volume was tested with alcoholic $FeCl_3$ solution. Presence of phenolic is indicated by a green color. The quantitative value of phenolic was obtained by finding the optical density at 230nm using the UV-VIS Spectrophotometer against the standard.

Detoxification Process: The detoxification of DOC has been done using three different methods as:

a. *Treatment with acetic acid*: In this process DOC was crushed and divided into 10 equal parts of 500 gm each. Mustard DOC was baked at 65°C and the moisture was maintained at 4% the product was then sprayed with acetic acid solution of concentrations 0.1-0.5%. The DOC powder was then kept overnight and on the next day it was subjected to mild steam and was again baked at same temperature for different time periods (1-3 hr) and final moisture level was maintained at 6.5%.

b. *Treatment with Ammonium hydroxide and ethanol*: In this process the material was immersed in a solution containing 10% ammonium hydroxide and 1% ethanol. The water and cake ratio was made to 1:10; the reaction mixture was kept at 80°C for half an hour. The material after separating from this solution was washed with fresh water. It took about 10 washings to properly remove maximum possible ethanol and NH_4OH mixture. The material was then centrifuged with industrial centrifuge to remove maximum possible water and was dried to 6% moisture level under hot air.

c. *Treatment with Saturated Steam:* This process was conducted by exposing the `DOC to the saturated steam at different pressures from 10-20 psi by employing autoclave unit after treating it with acetic acid. After treatment with steam DOC was washed with fresh water and then subjected to hot air oven to remove the water. Then DOC was dried for 2.5 - 3.0hr in oven as the moisture percentage came down to 4 -5% considering its storage view point.

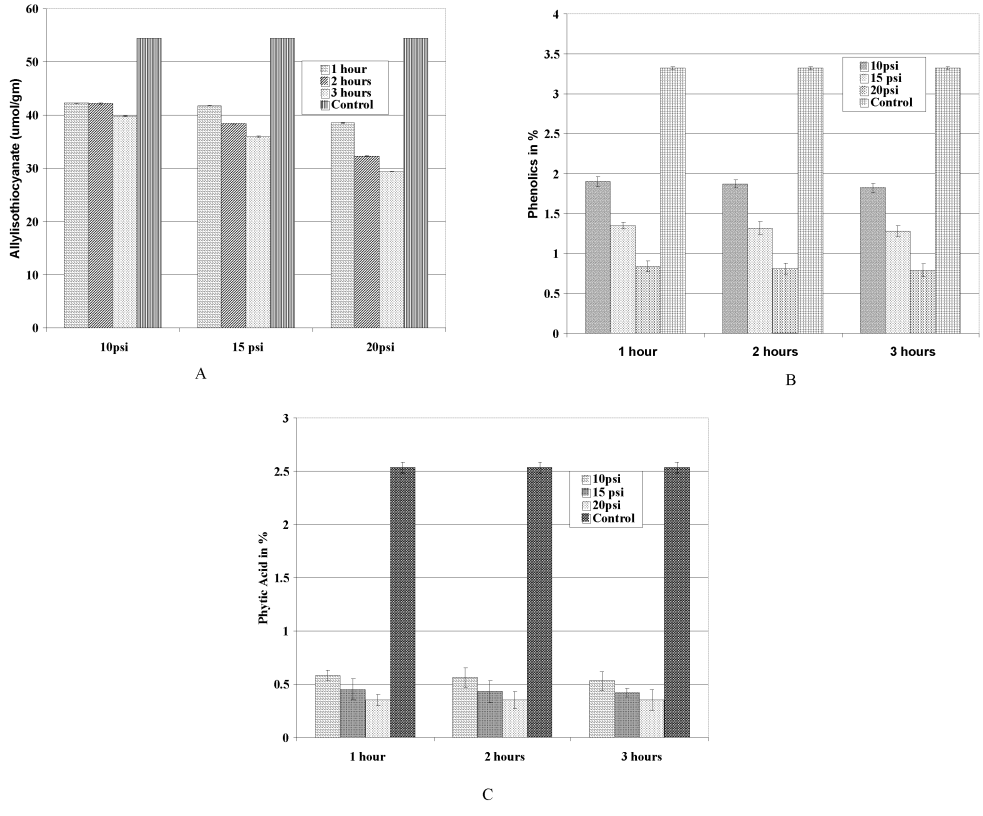

Fig.1. Amount of various antinutritional elements left in DOC after treatment with saturated steam and acetic acid treatment at different pressures. A. Amount of Allylisothiocyanate in µmol/gm, B. Phenolic % and C. Phytic acid % ; Fisher's test, $P < 0.05$, n (no. of samples) = 9.

Results

Acetic Acid Treatment: The phytic acid content was reduced to 1.91%, 1.90%, and 1.89% when baked for 1,2 and 3 hours respectively, the percentage of phenolic was found 1.75%, 1.74% and 1.71% respectively, allylisothiocyanate content in DOC also followed the same trend shown by phytic acid and phenolic. Its content after treatment for 3hr reduced to 28 µmoles from 52 µmoles per gram of DOC after drying.

Alcoholic ammonium hydroxide treatment: The amount of phytic acid in DOC was reduced to 2.11% of DOC, allylisothiocyanate reached the concentration of 41µmol/gm and DOC released about 50% of phenolic after the treatment with alcoholic ammonium hydroxide.

Saturated steam treatment: The amount of allylisothiocyanate left after treatment with acetic acid (0.5% of DOC) at 10, 15, 20 psi for 1, 2 and 3 hours in 9 different treatments were found to be 42.23, 42.11, 39.78, 41.72, 38.32, 35.85, 38.46, 32.19 and 29.33 µmoles per gm of DOC respectively. The amount of phenolic under the same treatment was found to be 1.90, 1.87, 1.82, 1.35, 1.32, 1.28, 0.84, 0.81 and 0.79% and phytic acid left in DOC after treatment was 0.58, 0.56, 0.53, 0.45, 0.43, 0.42, 0.35, 0.35, 0.35% with respect to the treatment.

Discussion

In present investigation three different treatments were tried on mustard DOC to make it palatable to livestock by reducing the content of various antinutritional elements. The first treatment of DOC was with acetic acid along with baking, which reduced the amount of various antinutritional elements up to great extent. This result is in accordance with Fallon (1995) who found the neutralization of phytic acid up to a great extent when treated with acetic acid. Colbin (1996) also observed the same results. Moist heat treatment drastically decreased the glucosinolate content (Schone et al., 1994). In second treatment process, the DOC was treated with ethanolic solution of Ammonium hydroxide that reduced the toxic materials abruptly. Our observations in this treatment got support with the findings of Shahidi et al. (1992) who determined the impact of this treatment on the reduction of allylisothiocyanate. Barlett (1998) also found reduction of toxicity in mustard meal after alkaline heating. In last experiment we treated mustard DOC with saturated steam at different pressures which resulted in comparatively a larger amount of removal of antinutritional elements without any visual loss in its texture or nutritional value ($P < 0.05$). This method was a slight modification of the method followed by Pawar and Palikar (1989) that soaked the pearl millet in water for reducing polyphenols and phytates.

Conclusion

In this study we tried to develop a commercially viable technology for detoxification of mustard oil cake by involving various methods either to neutralize or to remove the toxic substances so that it can be used as a high protein meal for livestock. We employed three treatments and all of them are efficient in removing the anti- nutritional elements but out of these we found the treatment of DOC with steam is the most suitable and commercially viable as it make the cake ready to eat feed for livestock and there is no need of huge investment in generating steam as the exhausted steam of the oil extraction unit can be used where as treatment with acetic acid make the food unpalatable and ammoniated alcohol treatment resulted in great loss of protein so is not commercially compliant. Moreover, the statistics of the results has shown that the treatment at 15 psi for 2hours is best as it does the detoxification maximally and is viable in terms of energy consumption.

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References

Barlett J. E., Klopfenstein C. F., Leipold H. W. (1998): Alkaline heating of canola and mustard meals reduces toxicity for chicks. Plant Foods for Human Nutrition **52**, 9-15.

Butler, E.J., Pearson A.W., Fenwick G.R. (1982): Problems which limit the use of mustard meal as a protein source in poultry diets. Journal of the Science of Food and Agriculture **33**, 866-875.

Colbin, A. (1996): Food and Healing, New York: Ballantine Books.

Dabrowski, K., Sosulski, F.W. (1984): Composition of free and hydrolysable phenolic acids in defatted flour of 10 oil seeds. Journal of Agriculture and Food Chemistry **32**, 128-130.

Delisle, J., J.Amiot, G. Goulet, C. Simard, G.J. Brisson, J.D.Jones. (1984). Nutritive value of Soybean, mustard and wheat flours in the rat Qual. Plant.-Plant Foods and Human Nutrition **34**, 243-251.

El Nockrashy, A.S., Mukherjee, D.K., Mangold, H.K. (1977): Mustard Protein Isolates by Countercurrent Extraction and Isoelectric Precipitation. Journal of Agricultural and Food Chemistry **25**, 193-194.

Fallon, S. (1995): Nourishing Traditions: The Cookbook That Challenges Politically Correct Nutrition and the Diet Dictorats: New Trends Pub Inc.

Graf, E. (1986). Chemistry and applications of phytic acid: An overview. Phytic Acid: Chemistry and Applications. E. Graf, ed. Pilatus Press, Minneapolis, MN, 173-194.

Krygier, K., Sosulski, F.W., Hogge, L. (1982): Free, esterified and insoluble phenolic acids. 2: Composition of phenolic in mustard flour and hulls. Journal of Agriculture and Food Chemistry **30**, 334.

Kumar R., Singh M. (1984): Tannins: Their adverse role in ruminant nutrition Journal of Agricultural and Food Chemistry **32**, 447.

Naczk, M., Shahidi,F. (1989) : The effect of methanol-ammonia-water treatment on the content of phenolic in canola. Food Chemistry **31**, 159-164.

Ohlson, R. (1978): Functional properties of Mustard oil and protein products: a survey. In: Proceedings of 5th International Mustard Congress, 152-167.

Pawar V.D., Parlikar G.S. (1990). Reducing the Polyphenols and Phytate and Improving the Protein Quality of Pearl Millet by Dehulling and Soaking. Journal of Food Science and Technology **27**, 140-143.

Shahidi F., Naczk M. (1992). An Overview of the Phenolic of Canola and Mustard. Chemical, Sensory and Nutritional Significance. Journal of the American Oil Chemists' Society **69**, 917-924.

Schone F., Kirchheeim U., Schumann W. (1994b). Glucosinolate degradation by mustard myrosinase and effect on mustard acceptability by growing pigs. Animal Feed Science and Technology **48**, 229-235.
Thompson D.B., Erdman J.W.Jr. (1982). Phytic Acid Determination in Soybean. Journal of Food Science **47**, 513-516.

Improvement of rapeseed meal quality via reduction of seed fibre-fractions

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Abstract

Rapeseed meal is an important product for animal and human nutrition. With drastically rising rapeseed production, an improvement of the quality and economic value of rapeseed meal is an increasingly important breeding aim. The meal possesses about 40% protein with a favorable composition of amino acids. However, due to the high contents of crude fibre and antinutritive components the utilisation of rapeseed meal is limited. To increase the possible use of rapeseed meal a reduction of antinutritive phenolic compounds through breeding of yellow-seeded oilseed rape varieties with thinner seed coats is of very high interest. Yellow seed colour is considered to coincide with low fibre content because the biochemical pathways leading to lignin and seed coat pigments have the same precursors. In this work fibre fractions are being measured in winter oilseed rape populations segregating for seed colour and in genetically diverse black-seeded materials. The major anti-nutritive fibre compounds in the oilseed rape seed coat, namely cellulose, hemicellulose and lignin, are quantified by extraction and measurement of the neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) fractions. Because these measurements are time-consuming and difficult to automate, the aim of this study is to develop near-infrared reflectance spectroscopy (NIRS) calibrations for high-throughput, non-destructive germplasm screening, selection and trait-genotype analysis of these important anti-nutritive rapeseed compounds.

Key words: Rapeseed meal quality - seed colour - fibre fractions - antinutritive compounds - near infrared spectroscopy

Introduction

Oilseed rape (*Brassica napus* L.) is the most important oil crop in Europe. Hence, rapeseed meal as residue after oil extraction is an important product for animal nutrition and a potential source for protein supply in human nutrition. Alongside the oil content and quality, due to the increasing production of oilseed rape the meal quality is becoming an increasingly important factor for the competitive character of oilseed rape. Rapeseed meal possesses about 40% protein with a favorable composition of amino acids; particularly the amounts of the essential sulphuric amino acids methionin and cystein are comparatively increased. Additionally, rapeseed meal is rich in minerals (Mg, P, Ca) and vitamins (tocopherols, cholin). However, the energy value of rapeseed meal as a stock food is limited by high proportions of fibre components, namely lignin, cellulose and hemicellulose, and antinutrive phenolic compounds, which are responsible for the decreased digestibility, the bitter taste, dark colour and the adstringency limited for monogastric animals (poultry and pigs). Major antinutritive and undesirable components in rapeseed meal besides glucosinolates are the phenolic acids, particularly sinapincholin (sinapin), the condensed tannins (proanthocyanidin) and lignin. To increase the quality of the rapeseed meal a reduction of antinutrive and undesirable fibre compounds is of very high interest. The breeding of yellow-seeded oilseed rape varieties is a promising opportunity in this regard, because the thin seed coats of yellow-seeded genotypes are associated with a considerable reduction in the condensed tannins and seed fibre content. Analogous phenotypes in *Arabidopsis thaliana* are collectively known as TT (*transparent testa*) mutants that in some cases regulate the thickness of the endothelium layer and subsequently lead to a thinner seed coat containing lower levels of lignin and proanthocyanidins. In particular, the transcription factors *TT1* and *TT16* are involved in regulation of endothelium cell development, while the endothelium-specific gene *TT10* is presumed to influence both lignin and proanthocyanidin biosynthesis genes in *A. thaliana*. (DEBEAUJON *et al.* 2003). Hence, these genes are interesting candidates for further progress in breeding yellow seeded low lignin and tannin rapeseed genotypes. Dehulling experiments of *B. napus* seeds leads to reduced tannin and fibre contents in different rapeseed varieties, meaning that antinutrtive tannins and fibre compounds are mainly localized in the seed coat (MATTHÄUS 1998). Additionally, different quality investigations of yellow seeded rapeseed varieties show that reductions of seed fibre and tannin contents lead to simultaneously increased oil- and protein contents (MARLES *et al*. 2004, BADANI *et al*. 2006). Since seed colour is very environmentally sensitive the use as a morphological selection marker is difficult. In this regard it is important to establish further analytic methods for screening of germplasm regarding improved seed quality. To determine the total seed fibre content and components the NDF (hemicellulose, cellulose and lignin), ADF (cellulose and lignin) and ADL (lignin) chemical detergent methods are used. However, these techniques are time-consuming and costly. Hence the main aim of this work is to develop low cost, non-destructive, high throughput screening techniques to measure total seed fibre contents. FONT *et al*. (2005) reported about the possibility to determine fibre fractions via near infrared spectroscopy (NIRS) in various *Brassica* species. NIRS is known as a desirable non-destructive high throughput method for the screening of large quantities of

breeding germplasm. With the development of new calibration equations for seed fibre and tannin a useful selection tool could be developed for the breeding of novel yellow-seeded rapeseed genotypes.

Material and Methods

In this study segregating doubled haploid (DH) populations derived from two crosses with different yellow seed sources were used to carry out chemical and molecular analysis. Seed samples were analysed in duplicate for NDF, ADF and ADL following the methods proposed by VAN SOEST (1964). To receive spectral information for the development of new NIRS calibrations with equations for fibre components, the same accessions were scanned in an NIR spectrometer (NIRSystems model 6500, Foss-NIRSystems) in reflectance mode. Spectra were acquired at 2 nm wavelength resolution over a wavelength range from 400 to 2500 nm.

Results and Discussion

First chemical analyses for the seed fibre fractions NDF, ADF and ADL were carried out for selected yellow, brown and black seeded DH-lines. This results showed a broad variation existing for the different seed fibre contents in the investigated rapeseed genotypes: NDF from 10.0 – 23.0, ADF from 5.0 – 13.0 and ADL from 1.0 – 6.0 % dry weight. The yellow-seeded lines showed significantly lower fibre contents than black-seeded lines. The chemical extraction of different raw fibre components showed that the content of ADF in the seed meal is largely influenced by the quantity of lignin (Figure 1), whereas the content of NDF is determined mainly by the amount of hemicellulose (Figure 2).

Lignin and hemicellulose, which are involved in secondary cell wall thickening, are considerably different in yellow and black seeded genotypes. The constituent fibre compounds derive from different biochemical pathways: Lignin is synthesised in the phenylpropanoid pathway and is therefore associated biochemically as a phenolic compound to the seed colour pigments (tannins, flavonoids). On the other hand hemicellulose is a branched polysaccharide. Thus, the synthesis and accumulation of lignin and hemicellulose are presumably influenced by different genes. For further investigations of major genes influencing the various fibre components QTL for the respective traits will be mapped using estimates based on the NIRS calibrations being generated in this study.

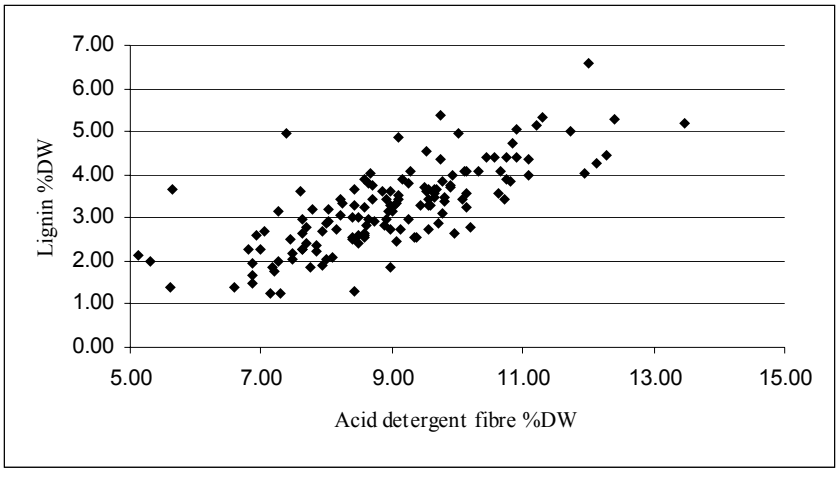

Figure 1: Scatter plot showing the correlation between contents of seed acid detergent fibre (ADF) and lignin (% dry weight). with a coefficient of determination of $R^2 = 0.5621$

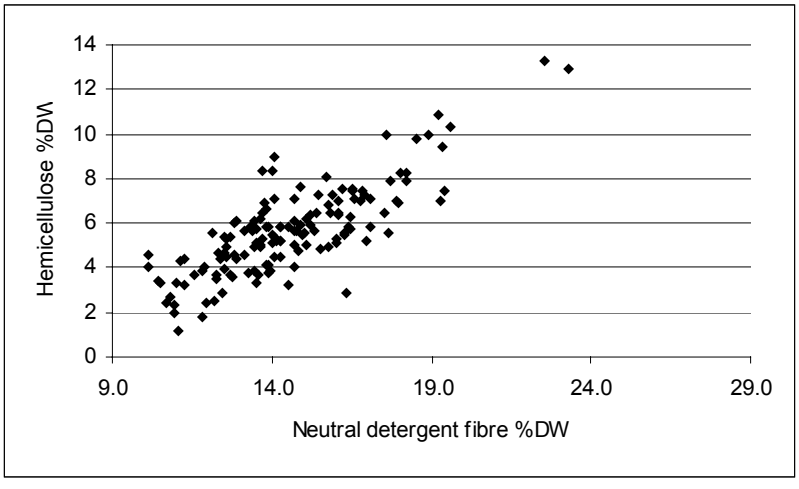

Figure 2: Scatter plot showing the correlation between the contents of seed neutral detergent fibre (NDF) and hemicellulose (% dry weight) with a coefficient of determination of $R^2 = 0.6463$

Dissection of QTL for fibre content into the different components – hemicellulose, cellulose and lignin - may also help to simplify the location of major genes involved in seed fibre content. QTL positions for fibre compounds will be compared with map positions of relevant genes for phenolic compounds, including structural and regulatory *transparent testa* (*TT*) genes from *Arabidopsis thaliana*. In previous work BADANI *et al*. (2006) reported that the seed colour is largely influenced by a major dominant locus on chromosome N18, with additional epistatic loci on N5 and N15. In the present study preliminary QTL analysis for individual fibre compounds and tannins revealed that these three QTL have significant effects on contents of lignin and ADF along with numerous important proanthocyanidins. On the other hand, numerous QTL were detected that do not have a significant effect on seed colour but presumably also contribute to the nutritional quality of the seed. Many of the genes that directly influence specific flavonoid and phenolic compounds are known in *Arabidopsis*, hence this information combined with comparative mapping of candidate genes should help to develop gene-linked selection markers for the breeding of low lignin and low tannin rapeseed genotypes.

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References

BADANI A. G., R. J. SNOWDON, R. BAETZEL, F. D: LIPSA, B. WITTKOP, R. HORN, A. DE HARO, R. FONT, W. LÜHS, W. FRIEDT, 2006: A model for the inheritance of seed colour in oilseed rape (*Brassica napus L.*) based on analyses of segregation data, QTL and associated quality traits in two genetically distinct crosses. Genome (submitted)

DEBEAUJON I., N. NESI, P. PEREZ, M. DEVIC, O. GRANDJEAN, M. CALBOCHE, L. LEPINIEC, 2003: Proanthocyanidin-Accumulating cells in *Arabidopsis* testa: regulation of differentiation and role in seed development. The Plant Cell 15: 2514-2531

FONT R., B. WITTKOP, A.G. BADANI, M. DEL RIO-CELESTINO, W. FRIEDT, W. LÜHS, A. DE HARO-BAILLON, 2005: Towards a global calibration for acid detergent fibre in rapeseed by visible and near-infrared spectroscopy. Plant Breeding 124: 410-412

MARLES S. & M.Y. GRUBER 2004: Histochemical characterisation of unextractable seed coat pigments and quantification of extractable lignin in the Bassicaceae. J. Sci. Food Agric. 84: 251-262

MATTHÄUS B., 1998: Effect of dehulling on the composition of antinutritive compounds in various cultivars of rapeseed. Fett/Lipid 100, 295-301.

VAN SOEST, P.J., 1964: Symposium on nutrition and forages and pastures: New chemical procedures for evaluating forages. J. Animal Sci. 23, 838-864.

The effect of different levels of rapeseed meals on broiler chick performance

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Abstract

In order the evaluation of different levels of rapeseed meals on broiler chick performance, the experiment was arranged in a randomized completely design with a factorial arrangement (4*2) and three replications. Four levels of rapeseed meals replacing to soybean meals including 0, 40, 60 and 80 percent (factor A) and two strains of broiler chick were including Arian and Ross (factor B). Broilers were growing to 56 days of age. The characteristics under investigation were average daily gain, feed conversion ration, moisture fecal (21, 42, and 56 days of age) and T3 and T4 Thyroids hormones (40 and 56 days of age) in each pen two males and females. At the end of trail one male and female of each pen were selected. The samples killed and dressing percentage heart, pancreas, liver, spleen, proventriculus, gizzard, gall bladder and intestinal weights were determined. The results indicated that 60 % rapeseed meals replacing to soybean meals for the feed conversion ratio and average daily gain were better than the other groups. The best body weight and dressing percentage related to group which had to 40 % rapeseed meal.

Key words: Rape seed meal, Soy bean meal, Broiler, performance, Thyroids hormones

Introduction

Canola is a plant which produces groups of yellow, four-petal led flowers. Canola is grown mostly in western Canada. It is the number one oilseed crop in Canada. Canola is Saskatchewan's second most important crop, after wheat. The word *canola* stands for "Canadian" and "oil" word production of rape seed meal/canola totaled 33.86 million tones or 13% of oilseed production (ERC 2001). The canola seed has following composition: whole canola seed contains high levels of lipid (approximately 55%) Ackman, 1990). For canola meal Sibbald (1997) indicated AME values ranging from 8.89 to 9.36 kcal/g. The goal of this research is to considering the effect of replacing different levels of rapeseed meals to soybean meals as a protein source.

Table 1: Composition of starter, grower and finisher diets (%).

6-8WKD	3-6WKD	0-3WKD	6-8WKC	3-6WKC	0-3WKC	6-8WKB	3-6WKB	0-3WKB	6-8WK[A]	3-6WK[A]	0-3WK[A]	Ingredient and composition(%)
63.56	62.30	51.08	65.6	64.41	56.48	67.64	66.46	58.95	70.81	70.46	63.86	Corn
10.56	10.85	13.38	13.28	13.87	16.77	16	16.50	20.17	21.42	22.18	26.97	Soybean Meal
17.14	17.74	21.32	12.86	13.31	15.99	8.57	7.87	10.66	0	0	0	Rapeseed meal
3	5	6.5	3	5	6.5	3	5	6.5	3	5	6.5	Fish meal
0.4	0.29	0.43	0.43	0.32	0.46	0.45	0.41	0.48	0.42	0.65	0.56	Dicalcium phosphate
0.98	0.93	0.77	1.02	0.92	0.83	1.06	0.97	0.88	1.18	094	0.97	Oyster shell
-	.	.	-	.	0.02	-	.	0.05	0.02	0.02	0.11	DL-methionine
0.5	0.5	0.5	0.5	0.5	0.5	0. 5	0.5	0.5	0.5	0.5	0.5	Vitamin-mineral premix
0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	Salt(sodium choloride)
3.66	2.19	2.92	3.12	1.63	2.26	2.58	1.09	1.61	1.46	0.05	0.32	Fat
												Total
												Chemical Analysis
3084.9	3000	2950	3084.9	3000	2950	3084.9	3000	2950	3084.9	3000	2950	ME (kcal/kg)
17.35	18.75	21.2	17.35	18.75	21.2	17.35	18.75	21.2	17.35	18.75	21.2	Crude protein (%)
0.77	0.84	0.92	0.77	0.84	0.92	0.77	0.84	0.92	0.77	0.84	0.92	Ca (%)
0.28	0.38	0.41	0.28	0.38	0.41	0.28	0.38	0.41	0.28	0.38	0.41	P (%)

A=Control group B=40% Rapeseed meal C=60% Rapeseed meal D= 80% Rapeseed meal

Material and methods

240 1-d-old commercial broiler chick (Ross and Arian strain) were weighted, distributed randomly to 4 treatments with 3 replicates., the experiment was arranged in a randomized completely design with a factorial arrangement (4*2) and three

replications. Four levels of rapeseed meals replacing to soybean meals including 0, 40, 60 and 80 percent (factor A) and two strains of broiler chick were including Arian and Ross (factor B). Broilers were growing to 56 days of age. Means compared with Duncan's Multiple Range Test at ($p<0.05$). The chicks were reared in deep litter on wood hulls in 1*1 pens. Feed and water were provided *ad libitum.* Body weights and feed conversion. The characteristics under investigation were average daily gain, feed conversion ration, moisture fecal (21, 42, and 56 days of age) and T3 and T4 Thyroids hormones (40 and 56 days of age) in each pen two males and females. At the end of trail one male and female of each pen were selected. The samples killed and dressing percentage heart, pancreas, liver, spleen, proventriculus, gizzard, gall bladder and intestinal weights were determined.

Results and Discussion

Feed Conversion Ratio: The results are presented in Table 2. Feed conversion data showed significant different ($p<0.05$). The results indicated that 60 % rapeseed meals replacing to soybean meals for the feed conversion ratio better than the other groups. The results are in agreement with the result of other researchers (Zeb *et al.,* 1999; Summers *et al.,*1988). Most probably this is due to presence of fishmeal, keeping amino acid moderation and no exchangein anion-cation balancing at the ration.

Table 2: The effect of rapeseed meals on feed conversion of different weeks.

Treatment	8-21 week	21-42 week	42-56 week	8-56 week
Control Group	2.45[a]	2.82[a]	3.09[ab]	2.81[a]
Replacing of 40% soybean meals by Rapeseed meals	2.59[a]	2.82[a]	2.56[a]	2.65[a]
Replacing of 60% soybean meals by Rapeseed meals	2.59[a]	2.83[a]	3.26[ab]	2.89[a]
Replacing of 80% soybean meals by Rapeseed meals	3.08[b]	3.41[b]	3.49[b]	3.33[b]

a,b means in the same column without a common superscript are significantly($p<0.05$).

Body weight: The results are presented in Table 2. There is no significant difference ($P>0.05$) in body weight between experimental groups in the comparison with control. The results are in agreement with the result of other researchers (Zeb *et al.,* 1999; Summers *et al.,* 1988). Most probably this is due to presence of fish meal, keeping amino acid moderation and no exchange in anion-cation balancing at the ration. Roth Maier *et al*. (1988) indicated that use of 5, 10, 15, 20 and 25 percents of full-fat Canola seed in the broiler ration has the negative effect on the chicken growth so that, body weight in experimental groups in comparison with control has showed 6.7-24% reduction. researcher has mentioned the decreasing of feed consumption is the cause of body weight decreasing (Sosulski, 1974; Roth Maier *et al*., 1988). According to Najib and Al-Khateeb (2004) with the exception of protein level, canola seed are very much similar to canola meal. High level oil in Canola seed in comparison to its meal, will cause meal and fish meal in starter diet and low level protein ratio, had adjusted feed consumption in experimental and control groups from 1 to 21 days of study. This issue do not support some results of researchers and with some other has conformity. This is in such a manner hat it has no conformity with the results of researches (Roth Maier *et al*., 1988; Lee *et al.*, 1984 and Nassar and Arscott, 1986). Roth Maier *et al*. (1988) used 5, 10, 15, 20 and 25 full-fat canola seed in the broiler diets, has observed that increasing proportion of Canola seed in the diet reduce continuously performance. No particular cause has been reported for decreasing of feed consumption yet, but the existence of phytic acid in canola seed and meal will cause reduction in calcium ability absorption and consequently, the feed consumption reduction (Semmers *et al.*, 1988). The results of this study supports other studies (Semmers *et al.,* 1988; Semmers *et al.*, 1977; Clark *et al.*, 2001). Zeb *et al.* (1999) reported that due to securing of amino acids in ration, feed consumption will not show any reductionby adding canola meal. Also, Hill (1979) has reported.

References

Ackman, R.G., 1990. Canola fatty acids-An ideal mixturefor health, nutrition, and food use. In: F. Shahidi(Ed.)Canola and Rapeseed, Production, Chemistry,Nutrition and Processing Technology. p: 81. VanNostrand Reinhold, New York.

Apata, D.F and V. Ojo, 2000. Efficacy of TrichodermaViride enzyme complex in broiler starters fedcowpea testa-based diets. In Animal Production in New millennium. Challenges and options. Proc. Of 25th NSAP Animal Conference, Michael OkparaUniversity of Agriculture, Umudike, p: 132-134.

Atteh, J.O. and F.D. Ologbenla, 1993. Replacement of Fish meal with maggots in broiler diets. Effects on performance and nutrient retention. Nig. J. Anim. Prod., 20: 44-49.

Bell, J. M., 1984. Starters fed cowpea testa -based diets. In Animal Production in New illennium. Challenges and options. Proc. of 25 NSAP Animal Conference, Michael Okpara University of Agriculture, Umudike, p: 132-134.

Banerjee, G.C., 1992. Poultry, 3 edn. Oxford and IBH pub. Co.Pvt. Ltd. New Dilhi, Bombay, Calcata.

Clark, W.D., H.L. Classen and R.W. Newkirk, 2001.Assessment of tail-end dhulled canola meal for use in broilers diets. Can. J. Anim. Sci., 81: 379-386.

Elwinger, K., 1986. Continued experiments with Newkirk, R.W. and H.L. Classen, 2002.The Effects of rapeseed meal of a Swedish low glucosinolate type fed to poultry. 2.An experiment with laying hens.

Swed. J. Agri. Res., 16: 35-41.Economic Research Service (ERS), 2001. Oil crops situation and outlook. OCS-2000, Oct. 2001. ERS,USDA, p. 66.

Hill, R., 1979. A review of the toxic effects of rapeseed meal with observation on meal from improved varieties. Br. Vet. J., 135: 3-16. Igwebuike, J.U., I.D. Kwari, C.O. Ubosi and N.K. Alade, 2001. Replacement value of spent sorghum grains for maize in broiler finisher diets. J. Sustain. Agri. Environ., 3: 224-233.

Lee, P.A., S. Pittam and R. Hull, 1984. The volutary food intake by growing pigs of diets containing treated rapeseed meals of extracts of rapeseed meals. Br. J. Nutr., 52: 159-164.

Leeson, S., J.D. Summers, 1997. Commercial Poultry Nutrition. Department of Animal and poultry Science, second edition,University of Guelph, Guelph, Ontario, Canada. ISBN: 964-91901-4-7.

Murphy, M., P. Uden, D.L. Palmquist and H. Wiktorsson, 1987. Rumen and total diet digestibilities in lactating cows fed diets containing full-fat rapeseed. J.

Dairy Sci., 70: 1572.
Mutzar, A.J.H.J.A. Likuski, and S.J. Slinger, 1978. Metabolisable energy content of tower and candle rapeseeds and rapeseed meals determined in two laboratories. J. Biol.Chem. 238: 235-237.
Mutzar, A.J. and S.J. Slinger, 1980. Apparent amino acid availabilityand apparent metabolisable energy values of Tower and Candel rapeseeds and rapeseed meal in two laboratories. Can. J. Anim. Sci., 58: 485-492.
Nassar, A.R. and G.H. Arscott, 1986. Canola meal for broilers and the effects of dietary supplement of iodinated casein on performance and thyroid status. Nutrition Report International. 34: 791-799.
Najib, H. and S.A. Al-Khateeb, 2004. The Effect of Incorporating Different Levels of Locally Produced Canola Seeds (*Brassica napus*, L.) In the Diet of Laying Hen. Int. J. Poult. Sci., 3: 490-496.
Nworgu, F.C, E.A. Adebowale, O.A. Oredein and A. Oni, 1999. Prospects and economics of broiler production using two plant protein sources. Trop. J. Anim. Sci., 2: 159-166.
Ojewola, G.S., A.S. Eburuaja, F.C. Okoye, A.S. Lawal and A.H. Akinmutimi, 2003. Effect of inclusion of grasshopper meal on performance, Nutrient utilization and organ of Broiler chicken J. Sustain Agri. Environ., 5: 19-25.
Raymer, P.L., D.L. Auld and K.A. Mahler, 1990. Agronomy of canola in the United States. p. 25–35. In: F. Shahidi (ed.), Canola and rapeseed: Production, chemistry, nutrition, and processing technology. Van Nostrand Rhienhold, New York.
Roth-Maier, A. Dora and M. Kirchgessner, 1988. Feeding of 00-rapeseed to fattening chicken and laying hens. Landwirtsch. Forschung, 41:140-150.
Salmon, R.E., 1984. True metabolisable energy and dry matter contents of some feedstuffs. Poultry Sci., 63: 381-383.
Sibbald, I.R. 1977. The true metabolisable energy values of some feedstuffs. Poult. Sci. 56: 380-382.
Sibbald, I.R. and K. Price, 1977a. True metabolisable energy values of the seeds of *Brassica campesyris, B. birta* and *B. napus*.Poultry Sci.56:1329-133.
Sibbald, I.R., 1977b. The true metabolisable energy values of some feedstuffs. Poult. Sci., 56: 380-382.
Singh, R.A., 1990. Poultry Production, 3rd edition.
Kalyany. Publishers, New Delhi, Ludhiana. Sosulski, F., 1974. Rapeseed Assoc.. of Canada 35 : 168.
Summers, J.D., S. Leeson and D. Spratt, 1988. Canola meal and egg size. Can. J. Anim. Sci., 68: 907-913.
Summers, J.D., S. Leeson, 1977. Effect of thyroxin and thiouracil addition to the diets containing rapeseed meal on chick growth and carcass composition. Poult. Sci., 56: 25-35.
Zeb, A., A. Satter and U. Meulen, 1999. Effect of feeding different levels of rapeseed meal on the performance of broiler chickens. Archive fuer Gefluegelkund, 63: 77.

FEED AND INDUSTRIAL RAW MATERIAL

Industrial Materials and Biofuel

Rapeseed for bio diesel production – international legal requirements and environmental benefits

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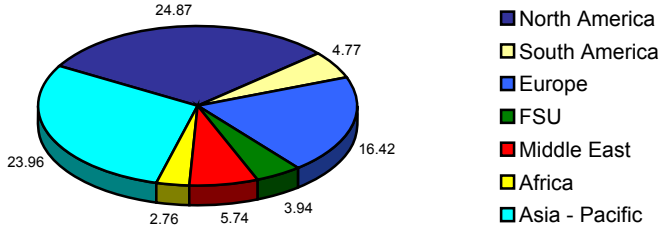
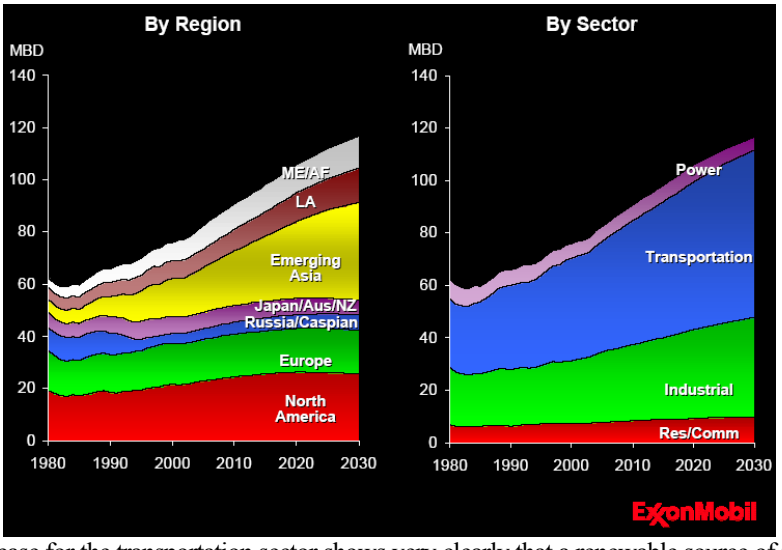
The earth contains a wide variety of carbon reservoirs that can be harnessed to meet society's power requirements, in the form of gaseous, liquid and solid fuels with liquid fuels having the most importance. The modern world has come to rely, almost exclusively, on fossil based reserves, a non-renewable resource, for the production of liquid fuels. The total consumption in 2005 was 82.46 m bbl/d with the following distribution between the different regions (source BP Energy Review 2006):



Asia will face a strong increase in its demand in the future and especially the transportation sector will increase dramatically.



The predicted increase for the transportation sector shows very clearly that a renewable source of fuel is required in order to meet the future energy needs of the world. The first generation of bio fuels replacing fossil fuels are bio ethanol and bio diesel.

Bio diesel is a renewable liquid fuel source that can be used as an alternative to petroleum diesel fuel. Bio diesel is 100 % soluble in fossil diesel and can be used in blends without any modifications of engines. The commercial bio diesel industry is a relatively new industry and the commercial market for bio diesel a young market.

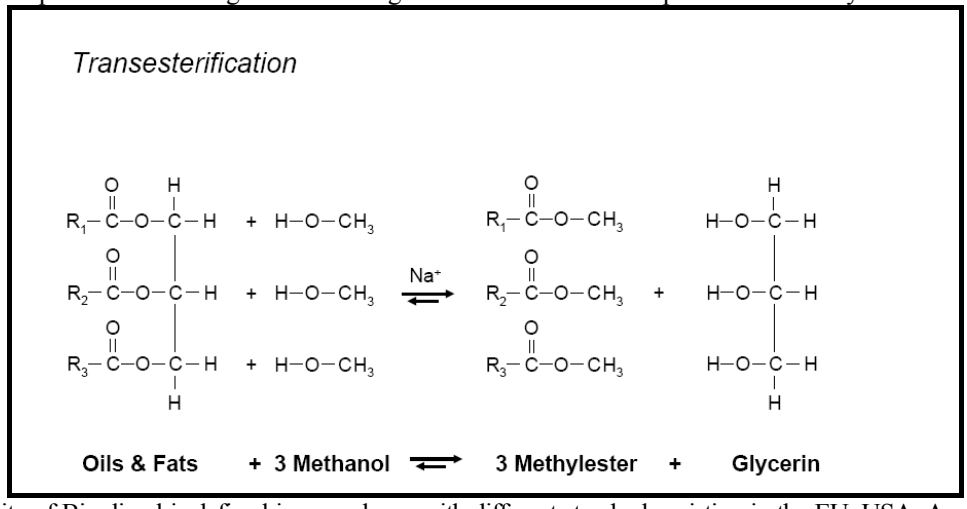
Vegetable oil, the raw material that bio diesel is made from, is stored solar energy in a high density and bio diesel has approximately 90% of the energy potential of petroleum diesel. The chemical formula is on average $C_{60}H_{120}O_6$.

From environmental performance standpoint bio diesel contents no heavy metals or sulphur and burns much more cleanly than petroleum diesel with reductions in most pollutant levels noted (carbon black: up to 50 %, PAH: up to 80%). Bio diesel has a nearly closed CO_2 cycle, since the combustion of bio diesel produces as much CO_2 as the plant consumes during

growing. Global climate change is one of the key concerns of the 21st century with serious implications for societies, environment and economies. The replacement of fossil fuel through bio diesel can help to avoid a lot of greenhouse gas emissions. Furthermore, in the case of a spill, bio diesel is a fairly environmentally benign chemical that is fully biodegradable. Conversely, petroleum diesel releases into the environmental are a serious threat to the ecosystems receiving these chemicals because many of the components of petroleum diesel are carcinogenic and persistent. Bio diesel is a promising alternative to petroleum diesel.

Additionally bio diesel can support the agricultural development in rural areas and is an important income for this profession.

Bio diesel is produced out of vegetable oil through transesterification in the presence of a catalyst.



The quality of Bio diesel is defined in accordance with different standards existing in the EU, USA, Australia, Canada, Indian, Korean and Brazil. Many other states are at the moment working on their standards and legal framework conditions for their respective countries.

The tables in annex I show a comparison of existing Bio diesel standards and their different quality criteria:

Rapeseed-oil represents, by far, the leading feedstock in Europe used for the constantly increasing Bio diesel production and this position has become even stronger with the further expansion of the European Union into the EU-25. Rapeseed-oil is the most important feedstock in Europe due to its following favourable properties:

- relatively high oxidation stability
- acceptable winter operability
- high yields of up to 2 t rapeseed oil/ha

Bio diesel out of rapeseed oil has very excellent cold flow properties. Therefore especially in countries with cold winters rapeseed is at the moment the most favourable feedstock, fulfilling all standards and the demand for rapeseed will continue to increase in the coming years.

Internationally there are different regulations for the implementation of bio fuels. The EU uses the mechanism to define market share targets for their member states. The EU member states use in their countries different national legislation for implementation like:

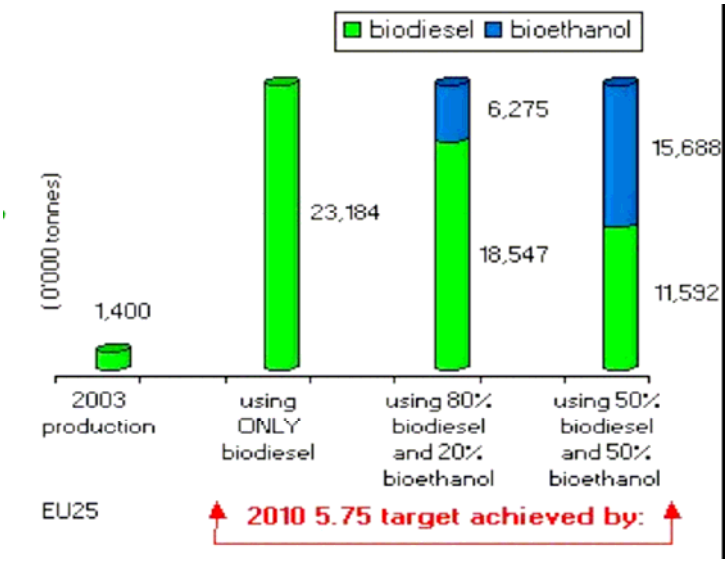
- Fuel tax breaks for biofuels
- Quota systems
- Substitution requirement for fuel suppliers/distributors
- Incentives to R&D in the field of biofuels
- Public procurement
- Tax reduction and incentives
- Capital grants for biofuel production facilities
- Awareness raising actions

Other systems are for example in USA where feedstock usage is subsidized and Australia where renewable energy usage is requirerd. Many countries are at the moment working on similar directives like the EU or thinking about possible regulations.

The EU released a directive for the use of bio fuels in May 2003, EU Directive 2003/30/EC "Promotion of the use of bio fuels or other renewable fuels for transport". This directive sets out national targets for bio fuels. Since October 2005 2% of the complete fuel consumption in the EU has to be substituted by bio fuels. The EU-directive regulates a continuous increase of substitution up to 5.75% in 2010. The market share shall be calculated on the basis of the energy content of all petrol and diesel used for transport purposes.

This fact will lead to a continuous increase in the demand for bio diesel in Europe. The EBB–European Biodiesel Board (source R. Garofalo, National Biodiesel Conference 2006, San Diego) estimates the following demand for bio fuels in Europe

at 2010 to fulfil the EU Directive 2003/30/EC:



Region/Country	European Standard EN 14214			US Biodiesel Standard ASTM D6751			Canadian Biodiesel Standard
Criteria	Test method	Unit	Treshhold value	Test method	Unit	Treshhold value	
Ester Content	EN14103	% (m/m)	> 96,5				Canadian General Standards Board supports ASTM D6751 or the European biodiesel standard EN 14214.
Cetane Number	EN ISO 5165		> 51	D613		⩾ 47	
Density (15°C)	EN ISO 3675, EN ISO 12185	kg/m3	860 - 900				
Sulphur Content	EN ISO 20846, EN ISO 20884	mg/kg	⩽ 10.0	D5453	mg/kg	⩽ 0.15	
Flashpoint	EN ISO 3679	°C	⩾ 120	D93	°C	130	
Cloud Point	EN 23015	°C	⩽ -2	D2500	°C	Report	
Pour Point	ISO 3016	°C	⩽ -9				
Linolenic acid methylester content	EN 14103	% (m/m)	⩽ 12.0				
Viscosity (40°C)	EN ISO 3104	mm2/s	3.50 – 5.00	D445	mm2/s	1.9-6.0	
Oxidation stability (110°C)	EN 14112	h	⩾ 6.0				
Copper strip corrosion (3h at 50°C)	EN ISO 2160		⩾ class 1	D130		⩽ No. 3	
Carbon residue (on 10% distillation)	EN ISO 10370	% (m/m)	⩽ 0.30				
Carbon residue (on 100% distillation)				D4530	% mass	⩽ 0.05	
Sulphated ash	ISO 3987	% (m/m)	⩽ 0.02	D874	% mass	⩽ 0.02	
Water content	EN ISO 12937	mg/kg	⩽ 300	D2709	% volume	⩽ 0.05	
Total contamination	EN 12662	mg/kg	⩽ 24				
Acid value	EN 14104	mg KOH/g	⩽ 0.50	D664	mg KOH/g	⩽ 0.80	
Iodine value	EN 14111	g Iodine/100 g	⩽ 120				
Polyunsaturated methyl ester (⩾4 double bonds)	EN 14103	% (m/m)	⩽ 1.0				
Methanol content	EN 14110	% (m/m)	⩽ 0.20				
Monoglycerid content	EN 14105	% (m/m)	⩽ 0.80				
Diglycerid content	EN 14105	% (m/m)	⩽ 0.20				
Triglycerid content	EN 14105	% (m/m)	⩽ 0.20				
Free glycerin content	EN 14105, EN 14106	% (m/m)	⩽ 0.02	D6584	% mass	⩽ 0.02	
Total glycerin	EN 14105	% (m/m)	⩽ 0.25	D6584	% mass	⩽ 0.24	
Alkaline metals group I (Na + K)	EN 14108, EN 14109	mg/kg	⩽ 5.0				
Alkaline metals group II (Ca + Mg)	EN 14538	mg/kg	⩽ 5.0				
Phosphorous content	EN 14107	mg/kg	⩽ 10.0	D4951	mg/kg	⩽ 10.0	
Distillation temperature, atmospheric equivalent temperature, 90% recovered				D1160	°C	⩽ 360	
Lubricity (50°C)							
Cold-filter plugging point			Regional specific				
Source:				G. Knothe (2004): The Biodiesel Handbook. AOCS Press			Canadian Renewable Fuels Association

Region/Country	Provisional Brazilian Biodiesel Standard ANP 255 (2003)			Indian Biodiesel Standard IS 15607			Korean Biodiesel Standard		
Criteria	Test method	Unit	Treshhold value	Test method	Unit	Treshhold value	Test method	Unit	Treshhold value
Ester Content					% (m/m)	> 96,5	EN 14078	% (m/m)	> 96,5
Cetane Number	EN ISO 5165		> 45			> 51			
Density (15°C)					kg/m3	860 - 900	KS M 2002	kg/m3	860 - 900
Sulphur Content	D5453	mg/kg	⩽ 0.10		mg/kg	⩽ 50.0	EN ISO 20846, EN ISO 20884	mg/kg	⩽ 10.0
Flashpoint	ISO/CD 3679	°C	⩾100		°C	⩾ 120	EN ISO 3679	°C	⩾ 120
Cloud Point					°C	⩽ -2			
Pour Point					°C	⩽ -9			
Linolenic acid methylester content					% (m/m)	⩽ 12.0			
Viscosity (40°C)					mm2/s	2.50 – 6.00	KS M2014	mm2/s	1.9-5.5
Oxidation stability (110°C)	EN 14112	h	⩾ 6.0		h	⩾ 6.0	EN 14112	h	⩾ 6.0
Copper strip corrosion (3h at 50°C)	EN ISO 2160		⩾ class 1			⩾ class 1	KS M 2018		⩽ class 1
Carbon residue (on 10% distillation)	EN ISO 10370	% mass	⩽ 0.50		% (m/m)	⩽ 0.05	EN ISO 10370	% (m/m)	⩽ 0.10
Carbon residue (on 100% distillation)									
Sulphated ash	D874, ISO3 987	% (m/m)	⩽ 0.02		% (m/m)	⩽ 0.02			
Water content	D2709		⩽ 0.20		mg/kg	⩽ 300	KS M 2115	% volume	⩽ 0.50
Total contamination					mg/kg	⩽ 24			
Acid value	EN 14104	mg KOH/g	⩽ 0.80		mg KOH/g	⩽ 0.50	KS M ISO 6618	mg KOH/g	⩽ 0.50
Iodine value	EN 14111	g Iodine/100g	⩽ 120			to report		no specification	
Polyunsaturated methyl ester (⩾4 double bonds)									
Methanol content	EN 14110	% (m/m)	⩽ 0.50		% (m/m)	⩽ 0.20	EN 14110	% (m/m)	⩽ 0.20
Monoglycerid content	EN 14105	% (m/m)	⩽ 1.00		% (m/m)	⩽ 0.80			
Diglycerid content	EN 14105	% (m/m)	⩽ 0.25		% (m/m)	⩽ 0.20			
Triglycerid content	EN 14105	% (m/m)	⩽ 0.25		% (m/m)	⩽ 0.20			
Free glycerin content	EN 14105, EN 14106	% (m/m)	⩽ 0.02		% (m/m)	⩽ 0.02			
Total glycerin	EN 14105	% (m/m)	⩽ 0.38		% (m/m)	⩽ 0.25	KS M 2412	% (m/m)	⩽ 0.24
Alkaline metals group I (Na + K)	EN 14108, EN 14109	mg/kg	⩽ 10.0			to report	EN 14108, EN 14109	mg/kg	⩽ 5.0
Alkaline metals group II (Ca + Mg)						to report	EN 14538	mg/kg	⩽ 5.0
Phosphorous content	EN 14107	mg/kg	⩽ 10.0		mg/kg	⩽ 10.0	EN 14107	mg/kg	⩽ 10.0
Distillation temperature, atmospheric equivalent temperature, 90% recovered									
Lubricity (50°C)							KS M ISO 12156-1Na	µm	⩽ 460
Cold-filter plugging point								no specification	
Source:	G. Knothe (2004): The Biodiesel Handbook. AOCS Press						Korea Petroleum Quality Institute		

The EU countries handle the implementation of this directive in different ways. The most effective way is to support bio diesel by compensating its higher cost (in comparison to fossil diesel) by removing the fuel tax, levied on fossil diesel, from bio diesel. At the moment this or the addition of additional taxes on fossil fuels that contain no bio diesel appears to be the most popular in Europe. Increasing fossil oil prices and the more economic production of bio diesel will lead to a more competitive price for bio diesel in the future.

Conclusion

Bio diesel like other bio fuels will show an increased demand in the coming years. Driving factors are the environmental benefits of bio diesel as well as the increase in demand and prices of fossil diesel. Bio diesel out of rapeseed shows favorable properties and is therefore especially in Europe widely used. Due to this fact the demand for rapeseed increased during the last years and can support the agricultural development in rural areas and is an important income for this profession.

Region/Country	Australian Biodiesel Standard 2003			New Zealand Biodiesel Standard NZS 7500:2500			Japan Biodiesel Standard	China Biodiesel Standard
Criteria	Test method	Unit	Treshhold value	Test method	Unit	Treshhold value		
Ester Content	EN 14103	% (m/m)	> 96,5		% mass	> 96,5	No Standard	No Standard
Cetane Number	EN ISO 5165		> 51			> 51		
Density (15°C)	ASTM D1298	kg/m3	860-890		kg/m3	860 - 900		
Sulphur Content	ASTM D5453, EN ISO 3675	mg/kg	⩽ 10.0		mg/kg	⩽ 50.0		
Flashpoint	ASTM D93	°C	⩾120		°C	⩾ 100		
Cloud Point								
Pour Point								
Linolenic acid methylester content								
Viscosity (40°C)	ASTM D445	mm2/s	3.5-5.0		mm2/s	2.0-6.0		
Oxidation stability (110°C)	EN 14112	h	⩾ 6.0		h	⩾ 6.0		
Copper strip corrosion (3h at 50°C)	ASTM DD130		⩽ No. 3			⩽ class 1		
Carbon residue (on 10% distillation)	EN ISO 10370	% mass	⩽ 0.30		% mass	⩽ 0.10		
Carbon residue (on 100% distillation)	ASTM D4530	% mass	⩽ 0.05		% mass	⩽ 0.05		
Sulphated ash	ASTM D874	% mass	⩽ 0.02		% mass	⩽ 0.02		
Water content	ASTM D2709	% volume	⩽ 0.50		mg/kg	⩽ 500		
Total contamination	EN 12662	mg/kg	⩽ 24		mg/kg	⩽ 24		
Acid value	ASTM D664	mg KOH/g	⩽ 0.80		mg KOH/g	⩽ 0.50		
Iodine value					g Iodine/100g	⩽ 120		
Polyunsaturated methyl ester (⩾4 double bonds)					% mass	⩽ 12.0		
Methanol content					% mass	⩽ 0.20		
Monoglycerid content					% mass	⩽ 0.80		
Diglycerid content								
Triglycerid content								
Free glycerin content					% mass	⩽ 0.02		
Total glycerin					% mass	⩽ 0.24		
Alkaline metals group I (Na + K)	EN 14108, EN 14109	mg/kg	⩽ 5.0		mg/kg	⩽ 5.0		
Alkaline metals group II (Ca + Mg)	EN 14538	mg/kg	⩽ 5.0		mg/kg	⩽ 5.0		
Phosphorous content	ASTM D4951	mg/kg	⩽ 10.0		mg/kg	⩽ 10.0		
Distillation temperature, atmospheric equivalent temperature, 90% recovered								
Lubricity (50°C)					µm	⩽ 460		
Cold-filter plugging point	TBA				no specification			
Source:	G. Knothe (2004): The Biodiesel Handbook. AOCS Press			B. Blackett (2006): Biodiesel developments in Auckland. Presentation at the "EECA Bio fuels Conference"			M. Shibuya (2005): A Study of Fuel Standards and Testing Methods for Biodiesel Fuel, presentation at "The First Japan-Korea Petroleum Technology Seminar"	

Processing-bioprocessing of oilseed rape in bioenergy production and value added utilization of remaining seed components

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Abstact

Cruciferous oilseed crops accumulate relatively high concentrations of oil, proteins and dietary fibres (DF) in their seeds, in addition to bioactive components as glucosinolates and myrosinase isoenzymes (thioglucohydrolase; EC 3.2.1.147). When mixed in the presence of moisture, myrosinase isoenzymes and associated components transform glucosinolates into various types of products, which reduces the value of the extracted oil and the remaining seed components, as well as producing unwanted environmental effects due to smell and toxicity. This gives a need for special care concerning myrosinase inactivation as the initial step during processing of oilseed rape, including technologies applied for biodiesel/bioenergy production. The myrosinase inactivation is thus a critical processing step, which needs to be performed at conditions with limited negative effects on other seed components, including proteins and glucosinolates. New bioprocessing technologies are now developed at levels that allow technology transfer from laboratory scale through pilot plant to industrial scale. The extraction of glucosinolates from the seed components remaining after oil separation-pressing and/or extraction is technically possible and has proven successful with the use of bioprocessing technologies. This is also the case concerning isolation of active myrosinases. The possibilities therefore exist for extraction and formulation of glucosinolates as "natural product derived" food and plant protection agents. With the great amounts of partly de-oiled rapeseed meal resulting from bioenergy/biodiesel production, the new bioprocessing technologies call thus for attention in relation to environmental friendly production of food (vegetable oil, protein and DF products), feed and other non food products.

Introduction

The current interest in cruciferous oilseed crops, especially double low oilseed rape, is mainly focused on the oil for uses both in the traditional areas of food, feed and non-food (GCIRC conf., 2003) and with increasing interest in biofuels- biodiesel (Inform AOCS, 2006, No.1). Quantitatively this oil is an important contributor to the total global production of vegetable oil, where it accounts for about 10 % and ranks third together with sunflower oil. Rapeseed oil is also the favoured feedstock for biofuels in EU, with a production of 3.2 MMT in 2005, and this figure is expected to be 6. MMT in 2006 (Inform AOCS, 2006, 17 (7)). Several EU countries contribute to this production, which currently is dominated by Germany followed by France and Italy (Inform AOCS, 2006, No.1). It is also claimed (Inform AOCS, 2006, No.1), that the future lies in the production of high quality biofuels from different kinds of feedstocks. We need thus to consider the opportunities for use of other cruciferous oilseed crops than double low oilseed rape. This can give a demand of SME-bioprocessing plants as supplement to the traditional oilmill processing units. In addition, the profitability of biofuel productions by SME and industrial oilmill processing plants may be determined by the added value of by-products (deoiled meal), rather than only the value of biofuel / biodiesel.

Following the production of large quantities of cruciferous oil, the result is the production of a great amount of deoiled meal, which has the potential to give added value products by appropriate applications (GCIRC conf. 2003). The chemical composition of cruciferous oilseed dry matter (DM) is approximately 40-45 % oil, 20-25% protein, 20-28 % dietary fibre (DF) and 5-15 % minor components, which reveals clearly the demand for utilization of the deoiled meal. The native components of double low oilseed rape, especially the oil and protein, have a high nutritive value if problems caused by unfavourable processing, storage and too high concentrations of antinutrients are avoided (Bellostas et al., this conference). The use of appropriate processing and/or bioprocessing procedures is also important for obtaining added value of production and use of cruciferous oilseed crops and products thereof.

Results and Discussion

The conventional processing procedures used by most oilmill industries are in a great part an adaptation of the technology used for soybean processing, which needs to be adjusted to the seed size, high oil content and the presence of glucosinolates characteristic to all cruciferous seeds. The crushing is then followed by heating to 90-110 °C for 15-20 min. in order to inactivate enzymes critical for the product quality i.e. myrosinase isoenzymes (EC 3.2.1.147) (Bellostas et al., this conference), lipase (EC 3.1.1.3) and lipoxygenase (EC 1.13.11.12). A varying part of the oil is then removed by pressing and most of the remaining oil in the resultant cake can then be extracted from flakes press cakes with petroleum ether/hexane using percolating bed extractors. Hexane is removed by heating under vacuum and the resulting meal can then be further treated

with steam to give the resultant rapeseed meal (Figure 1).

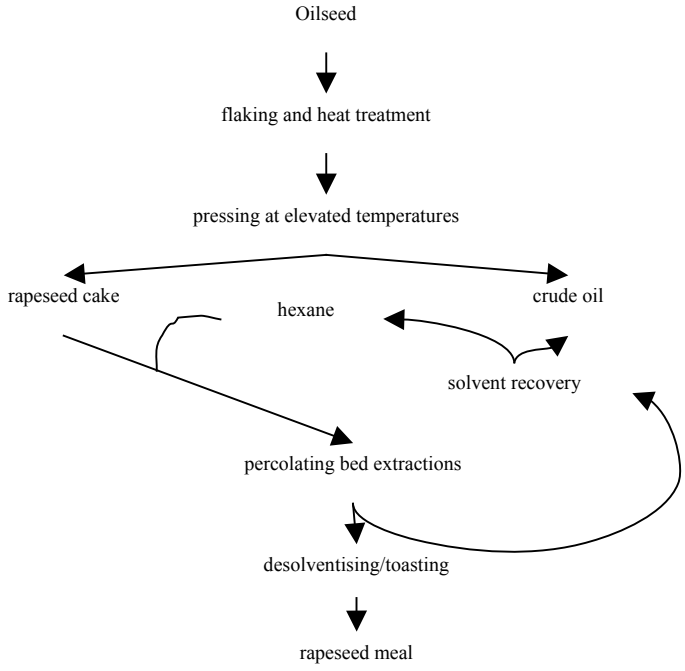

Figure 1. Flow diagram illustrating techniques often used for processing of oilseed rape in conventional oilmills.

The products obtained by the conventional oilmill processing are generally more or less dark, caused by degradation of seed components, including oxidation of some of the native rapeseed constituents. Dark coloured products are easily formed by oxidation of chlorophylls, carotenoids, phenolics and especially 4-hydroxyglucobrassicin (Jensen et al., 1991; Bjergegaard et al., 2001). Part of the other glucosinolates are also degraded and transformed into various products depending on glucosinolate type and processing conditions (Bellostas et al., 2007; Bellostas et al., this conference).

Some of the oxidation- and glucosinolate transformation products are lipophilic and will therefore be present in the oil fractions, which will result in unwanted additional sulphur- and bioactive compounds in the oil and in lipophilic membranes. The crude oil needs therefore refining before use. Volatile transformation products of glucosinolates will give unwanted smell, taste and environmental problems, but by air purification these problems can be solved and the compounds can thereby be used as wanted compounds (Bellostas et al., this conference).

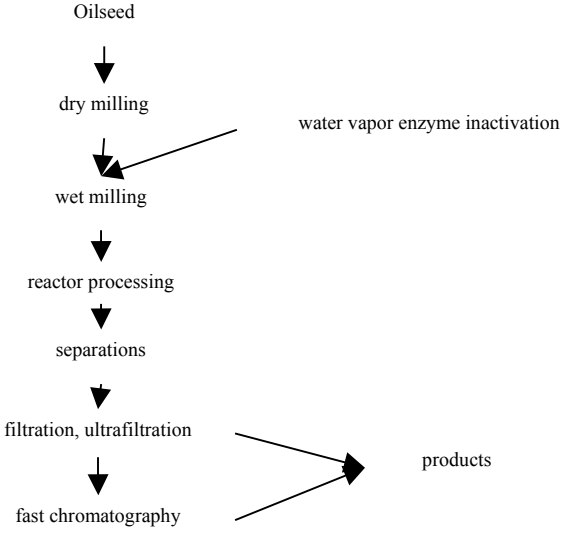

Figure 2. Flow diagram illustrating techniques used in Biorefining of cruciferous oilseeds.

The rapeseed cake and rapeseed meal obtained by conventional oilmill processing are mainly used as feed and for cattle feed as the dark colour does not create problems. The glucosinolates and glucosinolate products may especially create problems when too high concentrations occur in feed for monogastric animals (Bellostas et al., this conference). Both dark colour and glucosinolates or glucosinolate products may also create problems when the rapeseed meal is used for production of food ingredients and for added value non-food products (GCIRC conf. 2003); BOP project 1995-1999; and ENHANCE

project 1999-2003).

Biorefining – The Soft Processing Alternative for Processing of Cruciferous Oilseeds has been developed with basis in results obtained in the aqueous enzymatic processing project (Jensen et al. 1990) followed by the EU supported projects (BOP 1995-1999; Bagger et al., 1998; ENHANCE 1999-2003).

This biorefining process has been further developed as semi-industrial scale processing for biorefining of cruciferous oilseed crops based on cold pressing and/or whole seed extractions where SFE and or PSE can be included if or when needed for production of specific products as enzymes (myrosinase) and glucosinolates/glucosinolate products. The developed gentle and environmental friendly process ("Green Chemistry") does not include use of any petrochemicals, and thereby it provides the basis for production of high quality products. The processes have been developed owing to growing interest in obtaining high quality/added value products of the important oil and protein rich agricultural crops, especially double low oilseed rape, which is suitable for growth also in the northern countries in Europe. The aqueous based separation of the constituents in oilseed rape, with intact glucosinolates in well defined fractions, is considered to be a valuable supplement and alternative to traditional rapeseed processing.

This new type of biorefining, where glucosinolate degradation is avoided or strongly reduced, results in a yield of ca. 35% oil from the seed DM by the cold pressing step. This oil is of high quality with its natural content of antioxidants and without appreciable amounts of phospholipids and other constituents unwanted in the oil. Additional refining is principally not needed. Protein products with a high content of protein (60-80 %) can be produced in pilot plant scale, which, in addition to the protein contain especial DF and lipids originally present in the seeds. The concept includes possibilities for preparation of tailor-made specialty products.

Glucosinolates are isolated in separate fractions as are other seed components of interest. The extraction of glucosinolates and active myrosinase isoenzymes has proven successful with the use of bioprocessing techniques now developed. The possibilities hence exist for the extraction and formulation of glucosinolate products as environmental friendly food, feed and plant protection agents.

Liquid chromatographic methods including supercritical fluid techniques (Sørensen et al., 1999; Bjergegaard et al., 2003) are used for analytical process and product control, including oil, its content of chlorophylls, antioxidants, phytosterols, tocopherols, carotenoids and sinapoyl-derivatives.

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References

Bagger, C.L., Sørensen, H. and Sørensen, J.C.: High-quality oils, proteins, and bioactive products for food and non-food purposes based on biorefining of cruciferous oilseed crops. Plant Proteins from European Crops (J. Gueguen and Y. Popineau eds.) Springer Verlag (1998), 272-278

Bellostas, N., Sørensen, A.D., Sørensen, J.C. and Sørensen, H.: Genetic variation and metabolism of glucosinolates in cruciferous oilseed crops. In: Rapeseed Breeding: Advances in Botanical Research (Ed. Dr. Surinder Gupta) Academic Press/ Elsevier, Dan Diego, Vol. 54; 2007.

Bjergegaard, C., Buskov, S., Ib, K., Sørensen, H., Sørensen, J.C. and Sørensen, S.: Effects of various antioxidants and storage conditions on oxidative degradation of the indole glucosinolate 4-hydroxyglucobrassicin. In: Biologically-active Phytochemicals in Food. Analysis, Metabolism, Bioavailability and Function. The Royal Society of Chemistry, UK. ISBN 0-85404-806-5 (2001), 88-90

Bjergegaard, C., Buskov, S., Møller, P., Sørensen, H., Sørensen, J.C. and Sørensen, S.: Supercritical fluid techniques as methods of analyses for individual triacylglycerols and other lipids important for the quality of rapeseed oil. In: Proceedings of 11th International Rapeseed Congress, 6-10 July, Copenhagen, Denmark. ISBN 87-7611-003-6 (2003) PO6, 616-619. Inform AOCS, 2006, No. 1

Inform AOCS, 2006, No. 2

Inform AOCS, 2006, 17 (7)

Jensen, S.K., Michaelsen, S., Kachlicki, P. and Sørensen, H.: 4-Hydroxyglucobrassicin and degradation products of glucosinolates in relation to unsolved problems with the quality of double low oilseed rape. GCIRC - Congress, Saskatoon, Canada. (1991), V, 1359-1364.

Jensen, S.K., Olsen, H.S. and Sørensen, H.: Aqueous Enzymatic Processing of Rapeseed for Production of High Quality Products. In: Rapeseed/Canola: Production, Chemistry, Nutrition and Processing Technology (Ed. F. Shahidi) Van Nostrand Reinhold Publisher (1990), Chapter 19, 331-343.

Sørensen, H., Sørensen, S., Bjergegaard, C., Michaelsen, S.: Chromatography and capillary electrophoresis in food analysis. Royal Society of Chemistry, UK, (1999) 470 pp, ISBN 85404-561-9

A novel method for the preparation of biodiesel by transesterification of rapeseed oil using K_2O/γ-Al_2O_3 nano-solid-base catalyst

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Abstract

A novel method was developed for the preparation of biodiesel by transesterification of rapeseed oil and methanol using K_2O/γ-Al_2O_3 nano-solid-base catalyst. The influences of catalysts preparation conditions and transesterification conditions on the esterifiable ratio of rapeseed oil were studied. Under the optimum condition, the transesterification yield could achieve 94%.

Key words: biodiesel, nano-solid-base, rapeseed oil

Introduction

Biodiesel is obtaining more and more attention as an attractive fuel due to the depleting fossil fuel resources. The significance as a new energy source now results from its environmental benefits and the fact that it is made from renewable resources (Meher et al., 1984; Ayhan, 2003). Chemically produced biodiesel is mono-alkyl esters of long chain fatty acids derived from vegetable oils and animal fats by transesterification of triglycerides with methanol in the presence of catalysts (Xie et al., 2006; Zhang et al., 2005).

In the conventional method, the commonly used catalysts are sodium or potassium hydroxide, and a large amount of wastewater was produced to separate and clean the catalysts and the products. For the development of an environmentally benign process and the reduction of the production cost, heterogeneous catalysts have been studied in recent years (Kim et al., 2004; Sun et al., 2006). Compared with conventional solid base catalysts, nano-solid-base catalysts have been shown great advantages in transesterification (Zhu et al., 2004). The feed stock like rapeseed oil is widely planted all over China, and the "double zero" rapeseed oil is a new breed cultivated in HuBei province which is very suitable for the preparation of biodiesel. So it could be a promising feedstock in biodiesel production. Here it is reported an easy strategy to generate a novel nano-solid-base catalyst K2O/γ-Al2O3 derived from γ-Al2O3 supported KNO3 calcined in high temperature and is successfully applied to prepare biodiesel from rapeseed oil.

Experimental

Materials

Water free refined edible rapeseed oil is used in the reactions. KNO_3 and methanol are all analytical pure and the γ-Al_2O_3 is chemical pure of nano-grain.

Catalyst preparation

K_2O/Al_2O_3 nano-solid-base catalyst is prepared according to pestle-calcination method with some modifications (Zhu et al., 1996). Briefly, designed amount of KNO_3 was mixed with 10g nano-γ-Al_2O_3 powder, and then a certain amount of distilled water was added. After fully blended, the mixtures were decanted to evaporation dish which were then calcined in muffle for a certain time at the fixed temperature, therefore a series of K_2O/γ-Al_2O_3 nano-solid base catalysts were obtained. XRD and TEM methods were employed for the catalysts characterization.

Transesterification procedure

A 250ml three-neck glass flask with a water-cooled condenser and an automatic stirrer was charged with 50ml rapeseed oil, a certain amount of methanol and an appropriate amount of catalyst. The mixture was vigorously stirred and reacted for a required time at the fixed temperature. After the transesterification finished, the catalyst was filtered, and then the biodiesel could be separated from the glycerol with a funnel while the residual methanol was distilled from the liquid phase by decompression distillation. Biodiesel yield determination is based on the gas chromatography.

Results and discussion

The process of transesterification is affected by many factors. The main factors include the mode of catalysts preparation, such as loading ratio, calcination temperature and calcination time, and the transesterification condition such as the amount of catalyst in the reaction system, molar ratio of methanol to oil, reaction temperature and reaction time (Xie et al., 2006; Cui & Liu, 2005).

Influence of loading ratio of KNO3 on γ-Al2O3 on the transesterification

Fig.1 showed the influence of the loading ratio of KNO_3 on γ-Al_2O_3 on the transesterification yield. A maximum

transesterification yield was obtained at 70 wt. % KNO_3 loading ratio, and further increase of the loading ratio of KNO_3 led to the lower of the transesterification yield. The possible reason might be the formation of multilayer deposition due to the excessive amount of K_2O on the surface of γ-Al_2O_3, which could lead to the decrease of activated points (Zhu et al., 1997). So the suitable loading ratio of KNO_3 was nearly 0.7.

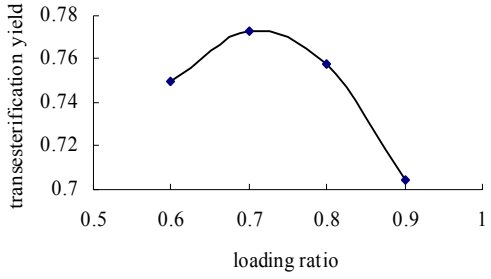

Fig.1. Influence of loading ratio of KNO_3 on γ-Al_2O_3 on transesterification yield (calcination temperature 600°C, calcination time 4h)

Influence of calcination temperature on *transesterification*

In the process of the solid catalyst preparation, calcinations of catalyst in high temperature makes for the reciprocity between carrier and activated ingredient which forms new crystal lattice, that is a new activated center, so the calcination temperature makes great importance to the catalytic activity. As illustrated in Fig.2, with increasing calcination temperature, the conversion was increased. Biodiesel conversion reached its maximum value at around a calcination temperature of 600°C. However, the conversion gradually decreased as the calcination temperature increasing beyond 600°C. It is maybe that higher temperature sintered the activated ingredient and lowered the catalytic activity (Meng & Xin, 2005). So the appropriate calcination temperature was 600°C.

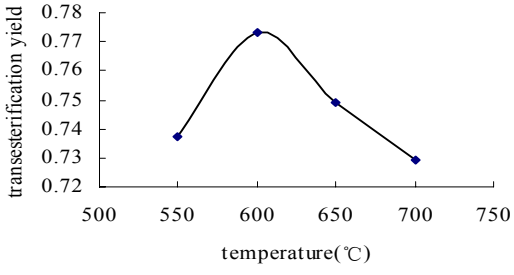

Fig.2. Influence of calcination temperature on transesterification yield (loading ratio of KNO_3 on γ-Al_2O_3 0.7, calcination time 4h)

Influence of calcination time on transesterification

The influence of calcination time on catalytic efficiency of the catalyst was shown in Fig.3.With prolonging the calcination time from 2h, biodiesel yield gradually increased, and when calcination time was very close to 3h, the maximum conversion is achieved, which indicated that KNO_3 had been totally decomposed. But continually prolonging the calcination time led to the dropping of the esterifiable ratio. The reason is maybe that the new-formed crystal lattice was destroyed when the calcination time is too long and it depressed the catalytic efficiency. So the calcination time was approximately 3h.

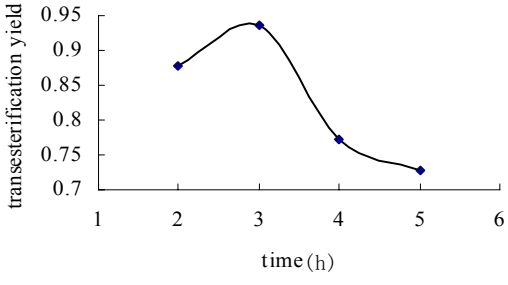

Fig.3. Influence of calcination time on transesterification yield (loading ratio of KNO_3 on γ-Al_2O_3 0.7, calcination temperature 600°C)

The main conditions influenced the esterifiable ratio, including the catalyst dosage, molar ratio of methanol to oil, reaction temperature, and reaction time. Here orthogonal experiment was adopted to analyze the influences of each factor and screen out the optimum reaction condition.

Table1 Design table of orthogonal experiment

Number / Factor	A	B	C	D
	Catalyst dosage (wt. %)	Molar ratio of methanol to oil	Reaction temperature (°C)	Reaction time (h)
Level 1	2	8:1	60	2
Level 2	3	12:1	70	3
Level 3	4	16:1	80	4

Table2 Intuitionistic analytic table of orthogonal experiment

Number / Factor	A	B	C	D	Yield
	Catalyst dosage (wt. %)	Molar ratio of methanol to oil	Reaction temperature (°C)	Reaction time (h)	
Experiment 1	1	1	1	1	0.640
Experiment 2	1	2	2	2	0.914
Experiment 3	1	3	3	3	0.804
Experiment 4	2	1	2	3	0.904
Experiment 5	2	2	3	1	0.898
Experiment 6	2	3	1	2	0.900
Experiment 7	3	1	3	2	0.916
Experiment 8	3	2	1	3	0.833
Experiment 9	3	3	2	1	0.936
Average value 1	0.786	0.820	0.791	0.825	
Average value 2	0.901	0.882	0.918	0.910	
Average value 3	0.895	0.880	0.873	0.847	
Pole difference	0.115	0.062	0.127	0.085	

From table 2, it was seen that the value of pole difference C>A>D>B, that was to say, influences reaction temperature>catalyst dosage>reaction time>molar ratio of methanol to oil. Choosing the maximum value of the average value of each condition, the optimum condition assemblence was $A_2B_2C_2D_2$, which was catalyst dosage 3wt. %, molar ratio of methanol to oil 12:1, reaction temperature 70°C, and reaction time 3h.

Increase of the catalyst dosage could promote the conversion of biodiesel. But too much base catalyst in the reaction system would lead to saponification reaction, which was unexpected (Liu et al., 2006). So the adequacy catalyst dosage of 3wt. % was chosen. The products of biodiesel and glycerin were difficult to separate if lacking of methanol, but properly increase of the molar ratio of methanol to oil to dilute the reaction liquid is feasible. Too much methanol didn't increase biodiesel yield indeed, also it is wasteful, and so the suitable molar ratio of methanol to oil could be 12:1. Esterifiable ratio increased markedly as the rising of reaction temperature. But when the temperature continually increases, the conversion decreased. The reason was maybe that the boiling point of methanol is 64.7°C, and higher temperature causes volatilization, which reduces the molar ratio of methanol to oil in the reaction system and goes against the process of transesterification (Li & Zeng, 2005). So the suitable reaction temperature was 70°C. As the reaction time goes by, the conversion of biodiesel increased also. The transesterification velocity was very rapid at the beginning of the reaction. When the reaction reached the balance point, the reaction speed slowed down gradually and got to the kinetics balance, which was similar to the kinetics process of homogeneous transesterification. The reaction time was chosen 3h from the results of the orthogonal experiment.

Comparison of general parameters of the quality of biodiesel with Germany Standard (DIN V51606)

Table 3 Comparison of general quality parameters of biodiesel with Germany Standard

Parameters	Biodiesel sample	Germany (DIN V51606)
Density (g/cm^3, 15 °C)	0.883	0.875~0.900
Kinematic viscosity (mm^2/s, 40°C)	5.0	3.5~5.5
Neutralization (mg/g, KOH)	0.317	⩽0.5
Sulphur content (wt. %)	0.007	⩽0.01
Ash (wt. %)	0.015	⩽0.03
CFPP (°C)	-5	⩽0

From table3, it is showed that the general quality parameters of the prepared biodiesel sample basically accord with Germany Standard (DIN V51606) (Han et al., 2002).

Conclusions

K_2O/γ-Al_2O_3 is an attractive and promising nano-solid-base catalyst for the production of biodiesel from rapeseed oil.

The catalyst with 70wt. % of KNO_3 loading on γ-Al_2O_3, which was calcined at 600°C for 3h, was found to be the optimum catalyst and gives the best catalytic activity in the transesterification reaction. When the reaction was carried out at a molar ratio of methanol to oil of 12:1, catalyst dosage 3wt. %, reaction temperature 70°C, and the reaction time 3h, the conversion of rapeseed oil to biodiesel can reach the maximum value of nearly 94%.

The easy preparation, favorable catalytic activity, mild reaction condition, shorter reaction time as well as the easy removal of solid catalyst for the transesterification emphasize the advantage of K_2O/γ-Al_2O_3 as nano-solid-base catalyst, and it is an environmentally benign process, which can minimize the production of pollutants.

References

Ayhan Demirbas. (2003). Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey. Energy Conversion and Management 44 (2003) 2093–2109

Bin Liang. (2005). Chemical Industry And Engineering Progress. 2005, 24(6) 577-585

Chengping Zhang, Jianming Yang, jian Lv. (2005). Industral Catalysis. May 2005, 13 (5) 9-13

Deqi Han, Dan Yuan,Jintao Wang, Huili Liu. (2002). Petro Chemical Technology economy. 2002, 18(4), 32-37

Hak-Joo Kim, Bo-Seung Kang, Min-Ju Kim, Young Moo Park, Deog-Keun Kim, Jin-Suk Lee, Kwan-Young Lee. (2004). Transesterification of vegetable oil to biodiesel using heterogeneous base catalyst. Catalysis Today 93–95 (2004) 315–320

Jianhua Zhu, Ying Wang, Li ShanKou. (1996). Chinese Journal of Catalysis (CUI HUA XUE BAO)July 1996, 17(4) 286-290

Jianhua Zhu, ying Wang, yuan Chun(1997). Chinese Journal of Catalysis. November 1997, 18(6), 498-502

Jun Zhu, Danbi Tian, Minglan Gu, Jingtang Wang, Yuanxiang Shi. (2004). Study on activity and preparation of nano catalyst KF/Al_2O_3. Journey of Shan Dong University, Jun.2004, 39(3), 88-91

Lin Bing Sun, Yuan Chun, Fang Na Gu, Ming Bo Yue, Qing Yu, Ying Wang, Jian Hua Zhu. A new strategy to generate strong basic sites on neutral salt KNO_3 modified NaY. Materials Letters 2006

Meher L.C., Vidya S.D., Naik S.N. (2006). Technical aspects of biodiesel production by transesterification-a review. Renewable & Sustainable Energy Reviews, Jun2006, vol. 10 Issue 3, 248-268

Shizhen Cui, chunshan Liu(2005). Industral Catalysis. July 2005, 13(7), 32-35

Wenlei Xie, Hong Peng, Ligong Chen. (2006). Transesterification of soybean oil catalyzed by potassium loaded on alumina as a solid-base catalyst. Applied Catalysis A: General 300 (2006) 67–74

Xin Meng, Zhong Xin. (2005). Petro Chemical Technology. 2005, 34, 282-286

Youyan Liu, Le Yu, Linfeng Huang, Tao Zhou. (2006). Modern Chemical Industry. Advances in applied research for biodiesel production. Apr. 2006, 26(4), 15-19

Yuqin Li, Hongyan Zeng. (2005). Journal of Huaihai Institute of Technology(Natural Sciences Edition). June 2005, 14(2), 45-48

Development of oilseeds for biodiesel feedstock in South Australia

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Abstract

In Australia, canola production has been approximately 1.5 million tonnes per annum over the past 6 years. Of this, about one quarter is crushed locally and three quarters is exported. In the past two years there has been an expanding interest in biofuels, particularly biodiesel with the potential for canola to be used as a feedstock. As well, over the last 25 years there has been a breeding program for mustard (*Brassica juncea*). While much interest has been in developing juncea canola as a food crop, there is also the possibility of using mustard in low rainfall areas for biodiesel production.

Production capacity of biodiesel from larger scale facilities is likely to reach over 500 ML by mid 2007 and there is also significant interest by individual farmers or farmer groups in the development of small scale facilities to produce their own fuel needs for farm operations. Costs of production for these small scale operations are likely to be a significant problem.

Issues that are likely to have an impact on biodiesel production from oilseeds include: cost of canola as a feedstock, variable production of mustard that is grown in low rainfall areas, ability to sell meal from the processing that may be done in regional centres, and government policies.

SARDI is undertaking a breeding program to develop oilseeds as a feedstock source for biodiesel production in South Australia and will aim to develop both mustard and canola for this use. Other oilseed options will also be investigated.

Key words: Biodiesel, *Brassica juncea*, Australia, canola, breeding, oilseeds

The Australian fuel industry uses over 15 billion litres of diesel per year, with the majority used in road transport, mining and agriculture. Demand for diesel is currently growing at about 3% per year. Over the past few years there has been a significant increase in interest in developing a biofuel industry in Australia to reduce the reliance on imported fuel. Significant investment has occurred in larger scale biodiesel production plants while many farmers are also investigating the feasibility of smaller on-farm production to turn their own crops into fuel. Costs of production for these small scale operations are likely to be a significant problem. In 2001 the Australian Federal government established a target of 350 million litres of biodiesel by 2010, but this is likely to be surpassed by 2007 when at least 500 million litres is likely to be produced. By 2010 it is likely that biodiesel production in Australia will be between 800 million and 1 billion litres per year. Biodiesel processing plants near most Australian capital cities are either operational, being built or are in the planning stage. In addition, plans are also being developed to increase the number of oilseed crushing plants which will also be built near capital cities and intensive livestock enterprises to make use of the increased meal production.

Much of the short term biodiesel production will be based on tallow, used cooking oil, palm oil and on oil from *brassica* crops. Each of these feedstock sources have issues to be considered if biodiesel production reaches the estimated 2010 figure. About 350,000 tonnes of tallow are exported from Australia at present and would be able to be processed into biodiesel. Used cooking oil is in limited quantities and collection and useage is limited. Palm oil will be used in biodiesel production in northern areas of Australia that are close to palm oil producing regions but use will be limited in southern areas due to biodiesel problems in colder conditions during winter. Canola oil produces very good quality biodiesel but is only able to be used in large amounts when the price of canola is at the lower limits or when mineral oil prices are at historically high levels. Canola production in Australia has averaged approximately 1.5 million tonnes over the past 8 years although the range has been from 2.3 million tonnes in 1999 down to about 400,000 tonnes in the drought of 2006. Of this, about one quarter is crushed locally and three quarters is exported. The other *brassica* crop that can be considered is *B. juncea*, that can be grown in lower rainfall areas.

Research into *B. juncea* in Australia has occurred over the past 25 years with the aim of developing an oil crop with equivalent oil quality to canola (Burton et al., 2003). *B. juncea* has many characteristics that should make it a viable crop in lower rainfall areas of Australia. These include good early vigour, early flowering, good blackleg tolerance, shatter tolerance and higher grain yields than canola when site yields are 1.2 t/ha or less. Both canola and *B. juncea* should have ready acceptance by farmers in lower rainfall areas as both crops have been shown to fit into cropping rotations and act as disease break crops in cereal production (Potter et al., 1997; Angus et al., 1999). Interest in *B. juncea* in Australia centres around three uses. The first is as a food crop equivalent to canola, secondly as a condiment crop and also as a possible feedstock for biodiesel. The first canola quality *B. juncea* cultivars, that will be used as a food crop, are expected to be commercialised in 2007. These cultivars will have low erucic acid, low glucosinolates and oleic acid levels of greater than 60%. However, these first cultivars will not have herbicide tolerance and so will need to be grown where broad leaf weeds are unlikely to occur. Future breeding efforts will be based on developing triazine tolerant and Clearfield *B. juncea* in the same way that canola

cultivars with herbicide tolerance have been bred.

Based on current rotations, if *B. juncea* could be grown on 10% of the total cereal growing area in the low rainfall winter cereal zones, the production area for Australia would be over 600,000 ha (Norton et al., 2005). In South Australia, we have estimated that up to 165,000 ha could be grown at maximum uptake of *B. juncea*. This area could produce average annual production of about 150,000 tonnes, fluctuating from 80,000 to 250,000 tonnes depending on seasonal rainfall. To produce this level of production it is likely that *B. juncea* would mainly be substituted for feed barley and land sown to pasture. Modelling by Primary Industries and Resources SA (PIRSA) suggests a 25-33% increase in average cropping gross margins in lower rainfall regions from the introduction of *brassica* break crops into the farming system.

SARDI has initiated a breeding program for *B. juncea* to develop this crop as a feedstock for biodiesel production. Initial breeding material was sourced from the Victorian Department of Primary Industries and a selection program began in 2003. Selection criteria have been earliness to flower, high grain yield, low glucosinolates and high oil content. Lines with lower levels of oleic acid have been used so as not to impact on the development of canola quality *B. juncea* which will be marketed as juncea canola. A breeding program has now begun in 2006 to enable a greater diversity of germplasm to be evaluated. While initial breeding will be with non-herbicide tolerant germplasm, efforts will be made to incorporate both triazine tolerance and imidazolinone tolerance into the *B. juncea* program. As much of the initial breeding over the past 25 years has been to develop canola quality characteristics, it is thought that more rapid improvement in grain yield is possible for *B. juncea* than for early maturing canola.

Conclusions

Significant investment in biodiesel production has occurred in Australia over the past two years. In southern Australia, oilseeds are likely to be a major source of biodiesel feedstock in the short to medium term. Issues that are likely to have an impact on biodiesel production from oilseeds include: cost of canola as a feedstock, variable production of mustard that is grown in low rainfall areas, ability to sell meal from the processing that may be done in regional centres, and government policies.

References

Angus J.F., Desmarchelier J.M., Gardner P.A., Green A., Hocking P.J., Howe G.N., Kirkegaard J.A., Marcroft S., Mead A.J., Pitson G.D., Potter T.D., Ryan M.H., Sarwar M., van Herwaarden A.F. & Wong P.T.W. (1999) Canola and Indian mustard as break crops for wheat. Proceedings of the 10th GCIRC International Rapeseed Congress, Canberra, 5pp

Burton W., Salisbury P & Potts D. (2003) The potential of canola quality *Brassica juncea* as an oilseed crop in Australia. Proceedings of the 11th GCIRC International Rapeseed Congress, Copenhagen, pp5-7

Norton R., Burton W. & Salisbury P. (2005) Agronomy for canola quality *Brassica juncea* in modern cropping systems. In: Potter T. (Ed), Australian Research Assembly on Brassicas **14**, 111-115, Port Lincoln, 2005. Proceedings. SARDI, Primary Industries and Resources South Australia

Potter T.D., Ludwig I. & Kay J.R. (1997) Brassica crops for rotations in low rainfall environments of South Australia. Australian Research Assembly on Brassicas Perth **11**, 128-132.

Determination of phase diagram of reaction system of biodiesel

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Abstract

Phase behavior of biodiesel reaction system in which rapeseed oil (RO) was converted into biodiesel by heterogeneous catalyst was investigated experimentally for improving the reaction rate and the efficiency of products recovery. Ternary phase diagrams of methanol, glycerol and methyl ester at various temperatures were determined. The results showed that the distribution coefficients (K_m) of methanol between biodiesel and glycerol phases were impacted by volume of glycerol and temperature significantly. It is suggested that the mass transfer limitation in the heterogeneous reaction of RO methanolysis can be broken on a certain extent by increase the ratio of methanol to oil and the temperature simultaneously, while the energy consumption for products recovery can be controlled in a multistage reversed flow process.

Key words: Phase diagram, biodiesel, heterogeneous reaction system

Introduction

Drainage area of Yangtze River is the biggest rapeseed-planting region in china. In recent years, double-low rapeseed planting area is enlarging quickly under the promotion of local governments. The boost of yield and the significant reduction of sulfur content make rapeseed become a potential source for bio-fuel production in china.

Generally, rapeseed oil (RO) is converted into biodiesel by transesterification with methanol; methanolysis is another term to imply this process. To improve the reaction rate and the product separation of methanolysis, data of phase behavior of the reaction system is essential (Zhou et al., 2006; Negi et al., 2006). In this system, the miscibility between methanol and triglycerides (oil) often plays as a bottleneck in the reaction, because their molecular polarities are quite different. In process of methanolysis, an induced period at the initiation of reaction was often observed, which indicate mass transfer limitation exist in this system (Barnwal et al., 2005; Meher et al., 2006). Different research groups took efforts for break the mass transfer limitation. Methanolysis performed at presence of cosolvents or enhanced by ultrasonic energy were reported (Cerce et al., 2005; Stavarache et al., 2005).

Heterogeneous catalysts for oil methanolysis show remarkable advantages in product separation and purification, but the reaction rate is lower compared to those of reactions promoted by conventional homogeneous catalysts (Bournay et al., 2005; Komers et al., 2001). In this work, the phase behavior of major components in the reaction system was investigated to improve the efficiency of heterogeneous reaction system.

Material and Methods

Refined RO was purchased from a local RO mill; chemical reagents for methanolysis of RO, preparation of solid base catalyst were analytical grade. Hexadecane (>99%) used in gas chromatograph analysis as internal standard was purchased from Tianjin research center of chemical reagent, China. Methods for determination of phase diagram, interfacial tension and product concentrations of biodiesel reaction system were as following:

Ternary phase diagram of methanol-FAME-glycerol: To preparation of rapeseed FAME, methanolysis of RO was carried out at 64°C for 60min using sodium methoxide as catalyst. The reaction product was settled in a separating funnel to remove glycerol, then the ester phase was washed twice by 80°C distilled water and dried at 95°C under vacuum. The final product was analysis by gas chromatography to ensure that the content of FAME was above 99% in mass fraction.

Methanol, rapeseed FAME and glycerol in different weight ratio were injected into screw-sealed glass tubes. These tubes were shaken intensively, and then kept in an isothermal water bath at specific temperatures for 24h to achieve phase equilibrium. Samples from upper phase and lower phase were withdrawn respectively to determine the content of glycerol and FAME.

The content of glycerol was determined by gas chromatography after glycerol in each sample was esterified completely with acetic acid using BF_3 as catalyst. The analysis was done using a gas chromatogram (GC9800, Kechuang, China) equipped with a flame-ionization detector. The GC column was a 30m × 0.32mm × 0.25μm PEG-20M capillary column (Chromatographic Technology Development Research Center, Lanzhou, China). Nitrogen gas was used as the carrier. The injector and the detector temperature were 230°C and 260°C, respectively. The column was kept at 150°C for 2min, then heated linearly from 150°C to 240°C at a rate of 10°C per min. Finally, the column temperature was kept at 240°C for 8min. Hexadecane was used as an internal standard. For FAME analysis, the same program was used.

According to the data of glycerol and FAME content in each sample, the composition of each phase could be calculated. By piloting data and the corresponding tie line, the ternary phase diagram was obtained.

Preparation of solid base catalyst: Porous magnesia obtained by calcination of sodium bicarbonate at 500°C was

impregnated in isochoric solution of acetic cadmium at the concentration of 12% for 6h, then the particles were collected to calcine at 700°C for 5h. Finally, calcine product was grind and sieved to obtain CaO/MgO solid catalyst with granularity at 80mashes.

Biodiesel production in heterogeneous reaction system: Heterogeneous reactions were applied to convert RO into biodiesel, two different processes were designed for comparison. The first was a one-stage process, the reaction was carried out with an initial catalyst concentration of 3%, an operation temperature of 60°C and with an alcohol/oil molar ratio of 6: 1. the reaction was stopped until the conversion rate of was achieved 98%. The second was a multi-stage process including several reversed flow. A scheme of the main reaction procedures is shown in Fig.1.

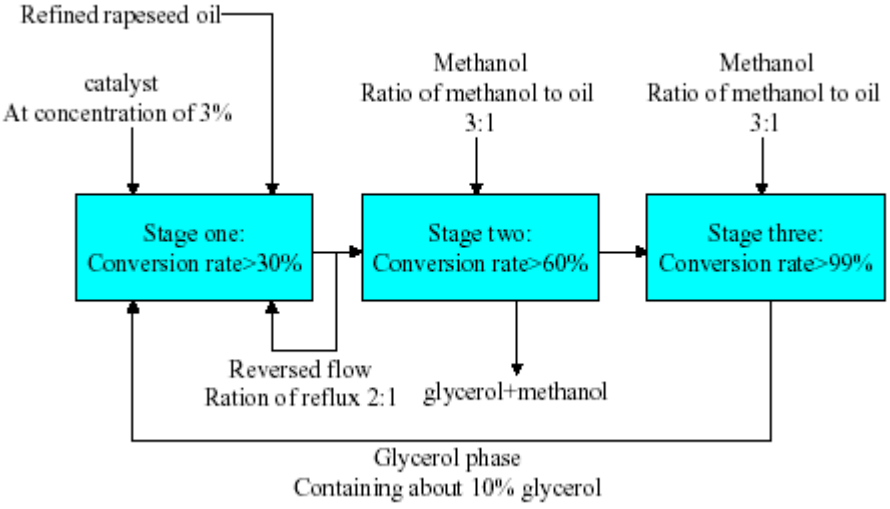

Figure1. Scheme of multistage reversed flow process

To evaluate the reaction rate of heterogeneous system, samples were withdrawn from the reaction system at intervals, mixed intensively with 0.1mol/L hydrochloric acid and settled in ice-water bath to terminate the reaction. Then hexane was used as solvent to extract FAME from the sample. The upper layer containing solvent and FAME was collected by centrifugation for gas chromatograph analysis. The conversion rate was calculated following the method described by She (She et al., 2005).

Results and discussions

Phase diagram of Methanol-FAME-glycerol. Reaction system for RO Methanolysis comprises reactants (triglycerides and methanol), intermediates (mono- and diglycerides) and final products (fatty acid methyl esters and glycerol), concentration of all components described above kept changing until the reaction reaches the end, therefore the process shows a very complex phase behavior. So we considered the oil of FAME as a single component, because of the molecular polarities of them is close compared to methanol and glycerol. Moreover, the influence of monoglycerides (MG), diglycerides (DG) and the solid catalyst was ignored in this study. Then phase diagram of Methanol-FAME-glycerol can be used to establish a simple but applicable description of the major relationships between each component in the reaction system.

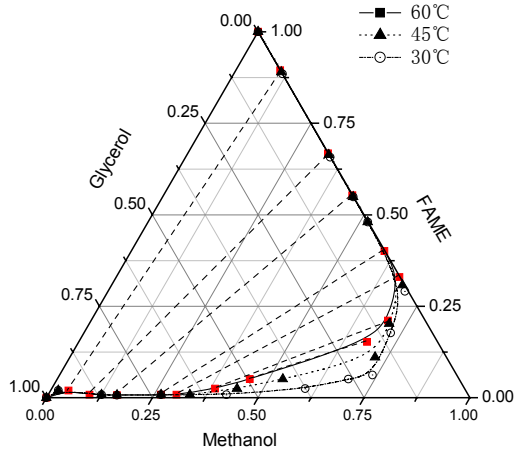

Figure 2. Ternary phase diagram of methanol-FAME-glycerol at different temperature

As shown in Fig.2, the phase diagram was divided into two parts by the joint curve of dots indicting the phase equilibrium data. The lower part denotes one liquid phase region; the upper part shows that two liquid phases will coexist. Temperature is a phase behavior effecting parameter can be manipulated easily for benefiting reaction or separation. According to the data in fig.2, higher temperature promotes solubilization of each component, enhances mass transfer and contact efficient of the reactants consequently. At lower temperature, the region of two liquid phases was larger. This phenomenon indicates that the separation of glycerol will be more complete at low temperature.

Distribution coefficient (K_m) of methanol. In reaction of methanolysis of RO, methanol not only serves as a reactant, but also acts as a solvent to facilitate mass transfer. Extreme sample reported was single-phase process developed for the transesterification of oil in a unique phase with the ratio of alcohol to triglyceride being 15:1 to 35:1. In commonly process in which alkali catalyst was used, excess methanol (generally at a molar ratio of 6:1) exists to switch the reaction to yield methyl esters. Therefore, there is more or less unconverted methanol should be recovered and reused in any process. Generally, methanol in glycerol phase is recovered by simple distillation; while methanol in FAME phase is removed from FAME by washing, rectification is used for methanol recovery, in which the energy consumption is much higher (Bouaid et al., 2005). From the energy-saving point of view, the distribution coefficient of methanol is an important factor.

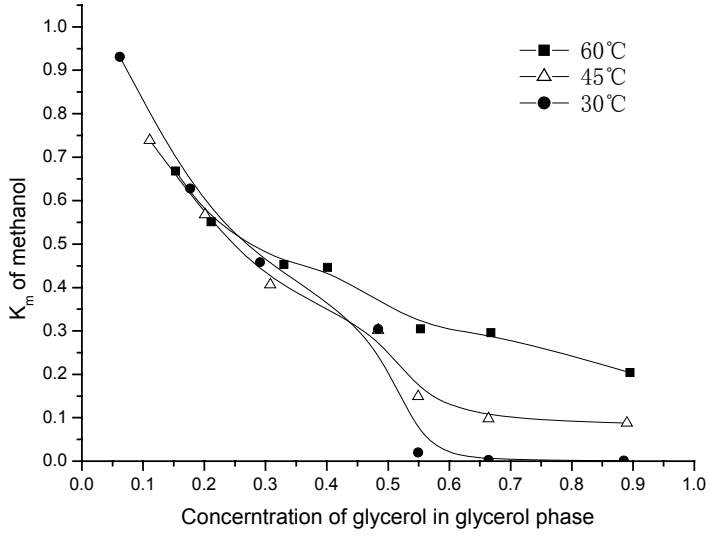

Figure 3. Distribution coefficient of methanol between FAME phase and glycerol phase

From Fig.3 it can be taken that the distribution coefficient (K_m) of methanol is temperature dependent. At a temperature of 30°C, unconverted methanol mostly existed in glycerol phase. It indicates that the hydrogen bonding between molecules of glycerol and methanol plays an important role in phase distribution. This phenomenon provides benefit for energy saving in methanol recovery, because simple distillation is enough for methanol-glycerol separation.

Comparison of one-stage process and multi-stage process. Preliminary studies for optimize the variables that effect the conversion rate of oil in heterogeneous reaction system was performed. Based on the result (data not shown) obtained in preliminary studies, we designed one-stage process. As shown in Fig.4, the reaction kinetics curve exhibited an S shape, in which the induced period lasted about 50min. It is indicated that the mass transfer limitation in heterogeneous system becomes a worse obstruct compared to those in homogeneous system. Increase the molar ratio of methanol to oil is an applicable measure to facilitate the reaction, but the energy consumption will increase at the same time. Concerning factors effecting the reaction rate, energy consumption and methyl ester yield, a multi-stage process was designed and evaluated as shown in Fig.5.

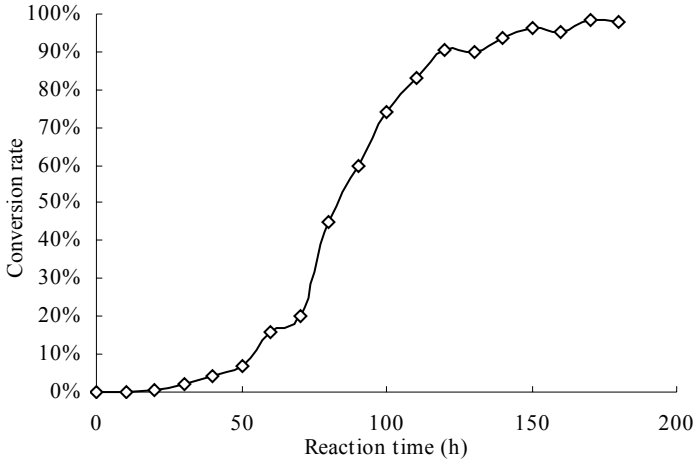

Figure 4. Reaction kinetics curve of one-stage process

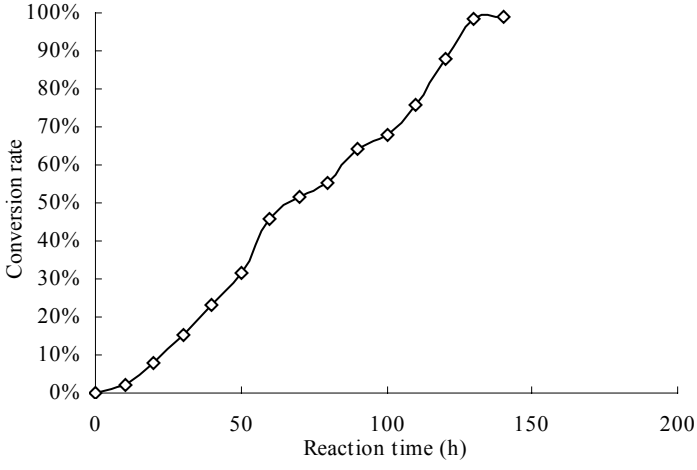

Figure 5. Reaction kinetics curve of multi-stage process

From the comparison of the reaction kinetics curve of each process, the multi-stage process shows several advantages as the following:

The reaction rate was increased. According to the kinetics curve, the induced period was reduced to 10min and the equilibrium time for reaction was reduced 40min.

The molar ratio of methanol to oil was reduced to 6:1; the unconverted methanol is mostly in glycerol phase. Therefore, the energy consumption for methanol recovery will be reduced.

The overall conversion rate of FAME will increased, it is due to the molar ratio of methanol to oil was reached about 30: 1 at the last stage of the process.

Conclusions

Temperature can affect solubility of oil in methanol and distribution of methanol between glycerol and FAME phase, it is a very useful parameter to enhance reaction or facilitate products separation. But at atmospheric pressure, the adjustable range is quite narrow. Multi-stage process provide another way to manipulate the reaction, different kinds of reversed flow can be chosen to change reaction balance or reaction rate, and to improve energy-saving. The multi-stage process in this study shows potential for industrial application.

References

Barnwal B K, Sharma M P. (2005). Prospects of biodiesel production from vegetable oils in India. Renewable and Sustainable Energy Reviews, 9, 363-378

Bouaid A, Diaz Y, Martinez M, Aracil J. (2005). Pilot plant studies of biodiesel production using Brassica carinata as raw material. Catalysis Today, 106, 193-196

Bournay L, Casanave D, Delfort B, Hillion G, Chodorge J A. (2005) New heterogeneous process for biodiesel production: A way to improve the quality and the value of the crude glycerin produced by biodiesel plants. Catalysis Today, 106, 190-192

Cerce T, Peter S, Weidner E. (2005). Biodiesel transesterification of biological oils with liquid catalysts: thermodynamic properties of oil-methanol-amine mixtures.

Komers K, Stloukal R, Machek J, Skopal F. (2001). Biodiesel from rapeseed oil, methanol and KOH 3. Analysis of composition of actual reaction mixture. Europe Journal of Lipid Science and Technology. 103, 363-371

Meher L C, Vidya S D, Naik SN.(2006). Technical aspects of biodiesel production by tranesterification—a review. Renewable and Sustainable Energy Reviews, 10, 248-268

Negi D S, Sobotka F, Kimmel T, Wozny G, Schomacker R.(2006). Liquid-liquid phase equilibrium in glycerol-methanol-methyl oleate and glycerol-monoolein-methyl oleate ternary systems. Industrial Engineering chemistry research, 45, 3693-3696

She Z H, Liu D C, Yu J H, Wu B. (2005). Determination of FAME conversion rate in methanolysis of *Jatropha curcas* L. oil. Process of Grain and Oil, 7,55-57

Stavarache C, Vinatoru M, Nishimura R, Maeda Y. (2005) Fatty acids methyl esters from vegetable oil by means of ultrasonic energy. Ultrasonices Sonochemistry. 12, 367-372

Zhou H, Houfang L, Liang B. (2006). Solubility of Multicompouent systems in the biodiesel production by transesterification of *Jatropha curcas* L. oil. Journal of Chemical Engineering Data, 51, 1130-1135

Oil seed rape as bio-diesel

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Abstract

Oil seed rape is the basic raw material for production of bio-diesel. The production of bio-diesel from oil seed rape is possible with higher production of oil seed rape on our plowed fields, by which the dependency on oil import would be reduced. Bio-diesel is the best alternative for oil. Lack of oil in European Union in the World is a big problem, as well as the pollution of environment due to the use of fissile oil. According to the estimations, the reserves of fossil oil, respectively, oil from the non renovated holes, are sufficient for the next 30-40 years. This means that significant shortage of oil can be expected in the next 15 years, followed by important price increasing (Kondić, J., 1998, 1999).

Because of that, Bosnia and Herzegovina must work more on improvement and increasing of oil seed rape production in the following period. With more use of genetic potential of varieties and hybrids. In order to achieve that, we must work intensively on the following: usage of new cultivated land under oil seed rape on state owned, as well as on privately owned sector, appliance of the contemporary soil management measures with new varieties, economic policy measures, etc.

Key words: oil seed, bio-diesel, production, Bosnia and Herzegovina

Condition and possibilities of oil seed rape in Bosnia and Herzegovina

Bosnia and Herzegovina has favorable agro-ecological conditions for higher and more lucrative production of oil seed rape, which represents comparative advantage. The climate is humid with optimal monthly average temperatures and total precipitation quantities during vegetation (Table 1. and 2).

In the past 24 years, the sown areas of oil seed rape in B&H have significantly varied from 5.000 ha in 1983 to 1.000 ha in 2005. Also The average yield varied from 1.00 t/ha in 1996 to 2.86 T/ha in 2004 (Table 3). At the same time variety and hybrid yield of oil seed rape in macro – trials were over 4 t/ha (Kondić J. 1988 i 1990).

Table 3. Condition of oil seed rape production in B&H

Year	In pre-war period		Year	In post-war period	
	Area (ha)	Yield (t/ha)		Area (ha)	Yield (t/ha)
1983.	5.019	2,15	1995.	1.364	2,00
1984.	4.817	1,84	1996.	1.201	1,00
1985.	5.423	1,68	1997.	1.749	1,20
1986.	4.246	2,19	1998.	705	1,50
1997.	4.848	2,30	1999.	767	1,60
1998.	4.201	2,18	2000.	30	1,40
1989.	4.155	1,82	2001.	-	-
1990.	3.210	2,37	2002.	-	-
1991.	-	-	2003.	49	-
1992.	-	-	2004.	141	2,86
1993.	10	-	2005.	1.000	-
1994.	1.621	2,20	2006.	-	-

Bio- diesel production

The interest for bio-diesel production has significantly increase, with higher crude oil price and in the world market, higher oil production from oil seed rape as well as from sunflower and soybean. The existing economic crises in our country related to the lower possibility of purchase of sufficient diesel oil quantities from the import, encourage the idea how to produce oil seed rape for processing in bio-diesel.

Bio-diesel fuel from the oil seed rape oil by the technological procedure esterification trygliceride of plant oils. The oil from oil seed rape and other oil plants are not suitable for diesel motor running because of the high ignition point (approx. 200°C) and other negative characteristics. Bio-diesel fuels derived from esterification have lower ignition point, nearly the same as the diesel fuel (60°C). Therefore bio-diesel fuel is methyl-ester fatty acid of plant oils which have similar physical-chemical characteristic to diesel fuel (D-2), (Đonlagić, 2002)

Bio diesel fuel is biological crude oil which does not pollute the environment because during fuel combustion there is no smoke, sulfur evaporations and other damaging fractions, and the biggest advantage its productions from renewed recourses. Now days, in the time of lacking and very expensive fuel from drill-holes, the possibility of our own bio-diesel production on

our plowed fields is of grate importance. Thus, agriculture would be freed from imported fuel dependency (Marijanović et. al. 2006).

One hectare of oil seed rape can give average yield of 2,5 t/ha, respectively 1,1 t/ha of oil seed rape oil. For production 1 t of bio-diesel, 1,12-1,14 t oil seed rape oil is needed. However, in Banja Luka area oil seed rape producers reached the yield up to 3 t/ha, and in the trials yield reached approx. 4 t/ha. With that kind of oil seed rape yields (3-4 t/ha), possible production of bio-diesel is approx. 1,5 t/ha bio-diesel. It is calculated that for performance of cultural measures at sowing certain crops, from 100-150 l/ha of diesel fuel is needed in average.

Therefore one hectare of sown oil seed rape can provide bio-diesel fuel for 7-11 ha of arable land.

Out of all oil plants, the oil seed reap oil is the most suitable for bio-diesel because it contains a good ratio of curtain fatty acid. It is very important that oil contains less important percentage of lower fatty acid which provides easier evaporation of methyl-ester on a working temperature of engine. That kind of bio-diesel has favorable-low ignition temperature, which is very important for the engine work especially in the winter period (Furman et. al., 1995, 2006).

Not all oil seed rape varieties equally suitable for bio-diesel production. The most favorable oil seed rape varieties are those which high content of erucic acid and low content glycosinolate.

Quality distribution of oilseed rape varieties

No.	Variety label	The content of erucic acid and glycosinolate
1.	++	Varieties with high content of erucic acid and glycosinolate
2.	0+	Varieties with low content of erucic acid and high content of glycosinolate
3.	+0	Varieties with high content of erucic acid and low glycosinolate
4.	00	Varieties with low content of erucic acid and glycosinolate.
5.	000	Varieties with low content of erucic acid, glycosinolate, row fibers and lower fatty acid

Double zero varieties (00) do not contain neither poisonous erucic acid in oil nor harmful glykosinolate in seed pellets. These varieties are the most cultivated ones currently. Single stranded – Zero – variety do not contain erucic acid in oil, but they have glycosinolate in seed pallets. The oil derived from the varieties that contain both erucic acid and glycosinolate (++), are used for technical purposes. **Varieties with (+0) label have high content of erucic acid and low glycosinolate and are the best for bio-diesel production**. The present methyl ester of erucic acid in bio-diesel does not an obstruction, and the side product of the seed pellets is suitable for cattle feeding because it does not have the harmful content of the glycosinolate.

Table 1. Perennial mean monthly temperatures (°C)(Meteorolgical stantion Banja Luka)

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	∑	average
1980	-1,5	02,9	07,6	07,9	12,7	18,6	19,9	20,1	15,6	11,6	04,2	-1,2	118,9	09,9
1981	-4,0	00,0	08,9	10,8	15,7	18,7	20,2	20,2	16,9	13,2	04,2	01,6	127,4	10,6
1982	-1,5	-0,1	05,4	07,9	16,8	21,0	21,6	20,2	19,0	12,9	06,2	05,3	134,7	11,2
1983	07,5	00,3	07,3	14,2	18,0	18,4	22,5	20,3	16,0	10,4	03,3	01,1	133,9	11,2
1984	00,7	00,4	04,6	10,0	15,0	18,0	19,5	18,7	16,6	12,4	06,7	01,3	124,1	10,3
1985	-4,6	-8,8	05,2	11,6	17,5	17,6	21,4	21,0	16,6	09,9	04,1	05,0	122,5	10,2
1986	01,3	-2,7	04,0	12,9	18,0	17,9	19,4	21,8	15,5	10,3	05,9	00,0	124,3	10,3
1987	-2,7	01,3	01,6	11,4	14,5	19,4	23,4	21,6	20,0	12,4	06,5	01,7	131,2	10,9
1988	04,3	04,4	05,5	10,5	16,4	19,1	23,2	21,4	16,3	10,7	01,5	01,8	135,1	11,2
1989	-0,6	05,3	09,6	13,3	14,6	17,6	21,0	20,8	16,0	10,3	05,2	03,2	136,3	11,4
80-89	-0,6	00,3	06,0	11,0	15,9	11,6	21,2	20,0	16,8	11,4	04,8	02,0	127,4	10,7
1990	00,8	06,7	09,4	10,9	16,6	19,2	20,5	20,5	15,1	12,3	07,4	00,5	139,9	11,6
1991	01,2	-1,1	09,2	08,4	12,2	19,6	21,8	20,3	17,7	09,6	06,9	-1,4	124,4	10,4
1992	00,9	03,9	06,9	12,1	15,8	19,6	21,4	24,6	17,0	11,3	07,7	01,6	142,8	11,9
1993	00,2	-0,4	05,1	12,3	18,1	20,0	21,2	21,2	17,0	13,1	02,3	03,4	133,5	11,1
1994	03,4	01,8	10,5	11,4	16,7	19,6	22,7	22,9	19,2	09,4	07,9	02,2	147,7	10,8
1995	00,2	06,5	06,0	05,8	15,4	18,5	23,3	19,9	15,5	11,9	04,7	02,1	129,8	10,2
1996	-0,7	-0,7	3,0	11,0	17,4	20,4	19,4	20,7	13,5	11,8	08,3	-0,7	123,4	10,2
1997	-0,7	03,8	06,0	07,2	16,9	20,6	20,7	20,3	16,1	09,2	06,8	03,2	131,5	11,0
1998	03,2	04,8	05,4	13,4	15,4	21,2	22,6	22,1	16,1	12,7	04,0	-2,6	138,3	11,5
1999	00,7	01,8	09,0	12,5	17,0	19,8	21,2	21,3	18,4	11,9	04,3	01,9	139,8	11,6
2000	-1,7	04,1	07,4	14,2	17,4	21,4	21,8	23,2	16,7	13,9	10,7	04,6	153,7	12,8
90-00	00,6	02,8	07,1	10,1	16,3	20,0	21,5	21,5	16,6	11,6	06,5	01,3	136,6	11,2
1980-2000	00,1	01,6	06,5	10,9	16,1	19,3	21,4	21,1	16,7	11,5	05,6	01,6	132,2	11,0

Table 2. Perennial precipitation quantities (Meteorological station Banja Luka)

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	∑	average
1980	66,5	55,6	98,2	122,5	192,0	144,0	36,2	93,3	57,6	81,4	196,0	137,8	1281,2	106,8
1981	112,9	64,5	137,6	57,7	77,9	149,5	46,8	48,0	106,5	70,8	80,8	172,4	1125,4	93,8
1982	27,0	13,7	118,7	77,1	39,4	112,4	103,3	90,5	49,8	102,4	54,3	206,9	995,5	83,9
1983	21,6	102,0	54,7	63,7	40,9	130,0	57,0	75,8	126,8	63,8	23,4	52,0	811,7	67,7
1984	135,6	86,8	112,2	76,9	137,6	126,1	106,3	158,3	97,2	99,7	80,3	28,2	1252,2	104,4
1985	69,5	37,4	122,9	149,5	46,2	91,8	33,0	94,6	27,3	34,6	161,6	66,8	935,2	80,6
1986	92,2	80,3	76,7	66,4	104,1	127,4	144,6	68,1	21,9	115,9	66,7	40,8	1004,7	83,7
1987	91,4	29,2	101,2	90,6	163,2	61,2	64,8	56,7	33,7	31,1	108,5	51,3	887,9	73,6
1988	96,9	75,5	123,4	58,9	83,3	111,4	25,5	75,3	91,7	60,9	66,9	50,8	924,9	77,1
1989	10,3	12,6	73,4	75,6	175,9	92,2	109,6	13,1	120,7	88,1	47,6	40,5	955,2	79,6
80-89	72,4	56,3	102,6	73,8	106,1	114,6	72,7	88,4	73,3	74,8	88,6	84,7	1008,3	84,0
1990	69,5	41,6	59,7	85,6	41,4	87,5	76,7	32,9	66,0	57,0	105,6	156,2	819,7	68,3
1991	50,4	41,8	91,6	125,8	171,9	90,9	273,4	64,9	39,7	181,1	134,0	41,0	1306,5	108,9
1992	20,2	45,1	102,5	77,2	36,1	181,7	81,9	06,4	49,5	197,6	155,8	61,1	1015,1	84,6
1993	49,5	29,3	135,8	55,7	47,5	107,1	106,7	106,8	160,4	79,2	149,2	143,3	117,5	97,5
1994	102,3	98,5	53,1	90,9	48,9	116,3	69,8	55,8	121,0	101,7	11,9	91,6	961,8	80,2
1995	148,7	74,3	128,3	68,8	70,6	163,8	38,6	126,9	120,2	02,1	69,2	137,0	1148,5	95,7
1996	33,4	56,5	107,2	63,5	203,4	76,1	60,3	53,6	225,0	67,0	165,6	97,1	1208,7	100,7
1997	81,7	74,5	59,0	122,1	90,1	87,7	103,4	103,5	43,8	116,7	124,5	139,3	1146,3	95,5
1998	108,1	08,8	50,1	83,3	82,7	104,3	81,1	37,4	167,3	102,0	99,3	69,0	993,4	82,7
1999	54,7	120,3	40,2	117,6	74,9	191,5	127,2	19,5	108,5	75,8	160,3	160,2	1250,7	104,2
2000	42,9	38,9	73,7	58,1	67,2	35,7	83,2	13,2	68,7	47,7	92,3	81,3	708,2	59,0
90-00	69,2	61,0	81,9	86,2	85,0	113,0	100,2	56,4	106,4	93,4	119,5	104,0	1075,2	89,6
∑ 80-00	1484.7	1232.7	1927,8	1686,3	1995,0	2387,7	1829,1	1503,6	1902,6	1776,6	2093,3	2024,1	21844,2	1040,2
sr.vr. 80-00	70,7	58,7	91,8	80,3	95,0	113,7	87,1	71,6	90,6	84,6	99,7	96,4	1040,2	86,7

Stead of the conclusion

Bosnia and Herzegovina has favorable agro-ecological conditions for lucrative oil seed rape production. There are all technological solutions and certain production experience for production of rape. However, there is a lack of necessary organization of producers and necessary stimulating measures of the organizers (bearers) of the production and government. Today, without the fuel the agricultural production is unimaginable. With higher shortage of fuel from holes, the significance of bio-diesel is increasing, because it is real alternative (replacement) for the oil. Bio-diesel does not pollute the environment as the oil does.

Europien Union gives grate importance to bio-diesel. The EU obligates its members to increase the bio-diesel production up to 5% out of the total use of common diesel fuel until 2006 and 12% until 2010. Therefore, for entrance to EU, certain quantities of bio-diesel must be produced in B&H as well.

Literature

Kondić, J. (1998): Industrial plants production (University book), Glas Srpski, Banja Luka

Kondić, J. (1990): The influence of the varieties and sowing time limits on oil seed rape yield in karst fields. Agricultural Gazette no. 6/90. Zagreb.

Kondić, J. (1999): Problems in villages and oil crops in Republic of Srpska and B&H. Agriculture and village in new conditions. Library B&H, development possibilities and perspectives. Sarajevo.

Furman, T., Nikolić, R., Tomić, M. i Savin, I. (2006): Bio-diesel, the chance and obligation of the agriculture 'Paper collection', volume 42, Novi Sad

Marjanović- Jeromila Ana, Marinković, R. i Furman T. (2006): oil seed rape as a raw material for bio-diesel production. 'Paper collection', volume 42, Novi Sad

Đonlagić, M. (2002): The main aspects of bio-diesel production and application. Agroznanje no. 2, Banja Luka

Rapeseed oil and biodiesel based diesel fuels: exhaust gas emissions and related health effects

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Abstract

Comparative exhaust gas studies were performed using Shell Middle Distillate (GTL) with lubrication additives; fossil diesel fuel (DF); rapeseed oil methyl ester (RME); premium diesel fuel (PDF – comprised of DF, RME and GTL); a blend of GTL and RME (B5GTL); and neat rapeseed oil (RO). In B5GTL, RME is used as lubricity improver. GTL and DF were included into the experiments in order to establish a relation of the results to existing standard fuels and future Gas-to-Liquid fuels, respectively. For the studies, a Mercedes-Benz (Euro 3) truck engine OM 906 LA was used in the ESC test. The regulated exhaust gas components, the particle size distribution as well as the mutagenicity of organically soluble fractions of the particulate matter were determined.

Overall, GTL consistently led to lower emissions than regular DF, while the particularly low nitrogen oxide emission and the significantly lower mutagenicity should be emphasized. RME showed advantages in the hydrocarbon, carbon monoxide, and particulate matter emissions. PDF joins these advantages but causes – like RME – high emissions of ultra fine particles. While in almost all values B5GTL showed the expected combination of GTL with little shifting towards RME, for PDF non-linear effects are observed. These include a lower particulate matter emission but also unexpectedly high mutagenicity, the causes of which must be studied further. RO exhibited in these tests the worst results: the highest NO_x and HC emissions, very high emissions of CO at idle, and a tenfold (!) higher mutagenicity than RME.

Keywords: Diesel engine emissions; rapeseed oil methyl ester; rapeseed oil; GTL; mutagenicity

Introduction

Growing demand on engine fuels for traffic purposes, the finiteness of fossil oil resources and rising environmental concern about health risks of diesel engine exhaust led to intense research activities over the last decades. Because of its efficiency and robustness the diesel engine became the dominating propulsion principle for trucks. The discussion about diesel exhaust related health effects led to a world-wide tightening up of exhaust gas regulations, especially for heavy duty vehicles. Apart from engineering efforts in the field of engine construction and exhaust gas treatment, fuel design has been established as another important tool to achieve improvements with respect to hazardous exhaust gas components.

As a prerequisite to market-wide usage of alternative fuels, their impacts on regulated and non-regulated engine emissions have to be evaluated. Minor or trace components of fuels could negatively affect engine or aftertreatment components and thereby lead to elevated emissions of harmful substances. Alternative fuels like biodiesel and rapeseed oil not necessarily have to be advantageous in terms of exhaust gas parameters, just because they derive from a renewable source. These questions have long been a research topic at the Institute for Technology and Biosystems Engineering (TB-BST) of the German Federal Agricultural Research Centre (FAL) in Braunschweig [1].

Therefore it was a substantial part of our investigations to compare a wide range of available fuels with respect to (HC, CO, NO_x, PM) and other not yet regulated exhaust compounds. Among the latter, ultra fine particles received special focus due to their suspected health risks to the respiratory and possibly blood system.

Materials and Methods

Studies were carried out at the emission test stand of the Institute TB-BST. A Mercedes-Benz Euro 3 engine OM 906 LA with turbocharger and intercooler was used; technical data are given in Table 1. As test procedure the 13-mode European Stationary Cycle (ESC) was chosen, cf. Figure 1.

Table 1: Technical data of Mercedes-Benz engine OM 906 LA

Piston stroke	130 mm
Bore of cylinder	102 mm
Number of cylinders	6
Stroke volume	6370 cm^3
Rated speed	2300 min^{-1}
Rated power	205 kW
Maximum torque	1100 Nm at 1300 min^{-1}
Compression ratio	17.4

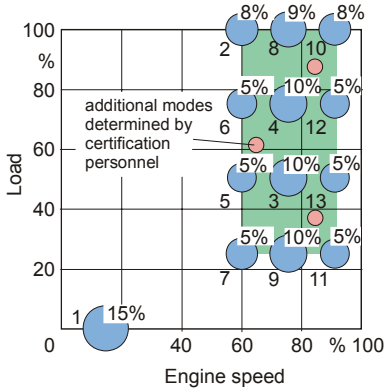


Figure 1. Modes of the 13-mode ESC test

Four neat fuels and two fuel blends were used:

DF	Fossil Diesel Fuel
RME	Rapeseed Oil Methyl Ester
RO	Rapeseed Oil (cold-pressed)
GTL	Shell Middle Distillate (SMDS) with additive (Gas-To-Liquid)
B5GTL	Blend (v/v): 5 % RME + 95 % GTL without additive
PDF	Premium Diesel Fuel, blend (v/v): 60 % DF + 20 % RME + 20 % GTL with additive

GTL (with and without lubricity improver) was delivered by Shell Research Ltd.; RME was provided by Oehlmühle Leer Connemann GmbH & Co. KG; DF by Haltermann Products BSL Olefinverbund GmbH in Hamburg. The so-called premium diesel fuel PDF and the blend B5GTL were blends of these fuels.

Regulated exhaust gas components carbon monoxide (CO), hydrocarbons (HC), and nitrogen oxides (NO_x) were sampled each second. A mean from three test runs was calculated from the corresponding values sampled in the last minute of each mode. Hydrocarbons were determined by means of a gas analyzer from Ratfisch Company (RS 55-T). Carbon monoxide was measured by means of an analyzer Multor 710 (Maihak Company). Nitrogen oxides were analyzed with a CLD 700 EL ht chemical luminescence detector from the EcoPhysics Co.

Particulate matter (PM) was determined gravimetrically after deposition to Teflon-coated glass fibre filters (T60A20, Pallflex, diam. 70 mm), with sampling intervals according to individual weighing factors of each engine mode. Weights of unloaded and loaded filters were determined to an accuracy of ± 1 µg by means of a microbalance M5P from Sartorius, always preceded by at least 24 hours of conditioning in a climate chamber held at 25 °C and 45 % relative humidity.

All particle size distribution measurements were accomplished after dilution of raw exhaust gas in a dilution tunnel. A dilution factor of about 10 is applied for determination of particle mass and Electronical Low Pressure Impactor (ELPI) measurements (aerodynamic diameter range 30 nm to 10 µm), an additional dilution by a factor of 7 is necessary to perform correct measurements via a Scanning Mobility Particle Sizer (SMPS) (with electrical mobility diameter range from 10 nm to 300 nm). Dilution factors were calculated from separate recordings of CO_2 contents in fresh air and diluted exhaust gas.

For mutagenicity testing, the *Salmonella typhimurium* / mammalian microsome assay, published by Ames et al. in 1975 [2], was applied. This study employed the revised standard test protocol [3] with the tester strains TA98 and TA100. Tests were performed with and without metabolic activation by a microsomal mixed-function oxidase system (S9 fraction). Preparation of the liver S9 fraction from male Wistar rats was carried out as described by Maron and Ames (1983) [3]. For induction of liver enzymes, phenobarbital and b-naphthoflavone (5,6-benzoflavone) were used instead of Arochlor-1254, which is a mixture of polychlorinated biphenyls (PCB) [4]. The mutagens methyl methanesulfonate (MMS), 3-nitrobenzanthrone (3-NBA) and 2-aminofluorene (2-AF) were used as positive controls. Immediately before use, the dried PM-extracts were dissolved in 4 mL DMSO, and the following dilutions were tested: 1.0, 0.5, 0.25, 0.125. The 2-AF was dissolved in 100 µg/mL and 3-NBA in 1 ng/mL DMSO, MMS was dissolved in distilled water (10 µg/mL). Every concentration was tested both with and without 4% S9 mix. Each extract was tested in duplicate. Tests were repeated during the following two weeks. The number of revertant colonies on the plates was recorded after 48 h of incubation in the dark at 37 °C. The background bacterial lawn was regularly checked by microscopy, as high doses of the extracts proved toxic to the tester strains, resulting in a thinning out of the background. Counting was performed by the use of an electronically supported colony counting system (Cardinal, Perceptive Instruments, Haverhill, GB). Results were considered positive, if the number of revertants on the plates containing the test concentrations was at least twice the spontaneous reversion frequency and if a reproducible dose-response relationship was observed [5].

Results

For DF and GTL, emissions of **total hydrocarbons (HC)** were about 40-fold below the Euro 3 limit (Fig. 2). RME caused a further reduction of HC. In contrast, rapeseed oil showed a considerably higher emission. B5GTL emissions fairly well reflected relative amounts of neat components in this blend, while PDF led to higher than predicted emissions.

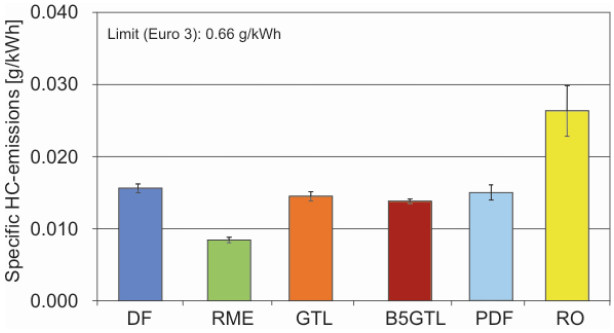


Figure 2 Specific HC emissions of different fuels, ESC test, OM 906 LA engine

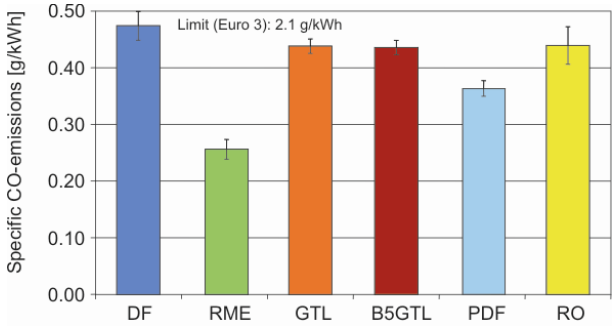


Figure 3 Specific CO emissions of different fuels, ESC test, OM 906 LA

Carbon monoxide (CO) (Fig. 3) was found to be well below Euro 3 limits by a factor of at least 4 for all fuels including RO. RO, however, exhibited very high CO emissions in idle mode (600 ppm). RME again gave best results since specific emissions were lower by a factor of about 8 than the limit value of 2.1 g/kWh. B5GTL caused only slightly reduced CO emissions, fairly well predictable from relative amounts of components. Data for PDF, in contrast, deviated from the calculated value (0.42 g/kWh) towards an emission benefit.

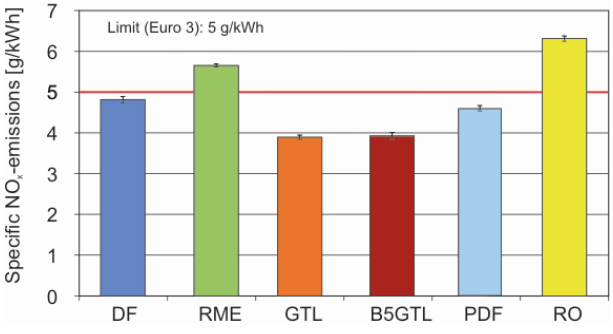


Figure 4 Specific NO_x emissions of different fuels, ESC test, OM 906 LA

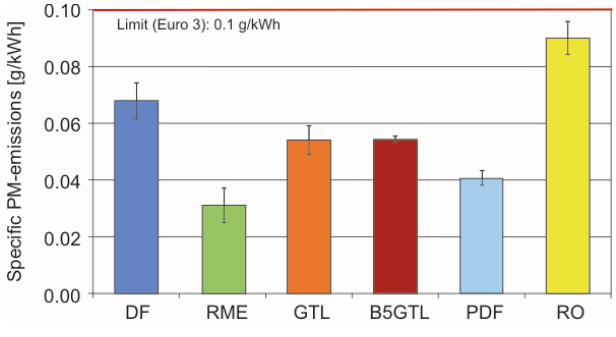


Figure 5 Specific PM emissions of different fuels, ESC test, OM 906 LA

Regarding **nitrogen oxides (NO_x)** RME and RO exceeded the Euro 3 limit of 5 g/kWh, whereas the other fuels remained just below it (Fig. 4). Lowest emissions were obtained with GTL and B5GTL, the latter showing linear behaviour in the sense of a small NO_x increase corresponding to its 5 % RME content. Premium diesel fuel profited above average and gave lower emissions (4.8 g/kWh) than the theoretical value calculated from results for the neat components.

Emissions of total **particulate matter (PM)** remained below the Euro 3 limit of 0.1 g/kWh. GTL, B5GTL and PDF showed a significant reduction of emissions by about 20 % compared to DK. The reduction by neat RME was even larger – about 55 %. Data for rapeseed oil were much higher, but remained just below the limit. While no difference was observed between B5GTL and GTL, PDF featured a positive effect: PM remained about 30% lower than the calculated 58 mg/kWh (Fig. 5).

The investigations on fuel blends were performed in two temporally separate campaigns with the important consequence that ambient temperatures differed by more than 20 °C. While this did not affect legally regulated components, **particle size distributions** were changed to some extent. ELPI data (determination of aerodynamic diameters down to 28 nm) can be regarded as almost consistent between both measurement campaigns (Fig. 6). SMPS data (determination of electrical mobility diameters down to 10 nm) in contrast showed distinct differences, possibly caused by the necessary installation of the second dilution tunnel. Due to this fact these two measurement series are depicted in separate diagrams (Figs. 7, 8).

Measurement of aerodynamic diameters by ELPI revealed the results summarized in Fig. 6. Particle size distributions of DF and GTL showed almost no differences. For RME, the shapes of distribution curves followed the same pattern, but covered considerably smaller particle numbers. Fuel blends led to expected (i.e. predictable from their composition) particle counts and curve shapes, with the important exception that B5GTL emitted less than expected over the range from 28 to 55 nm.

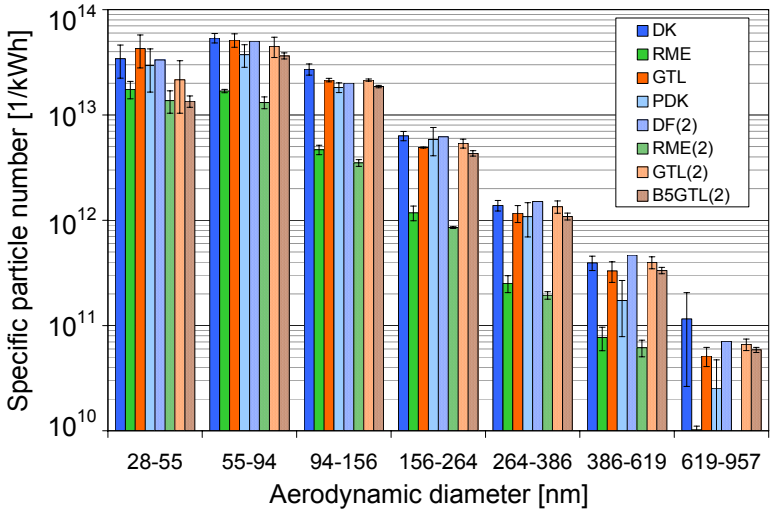
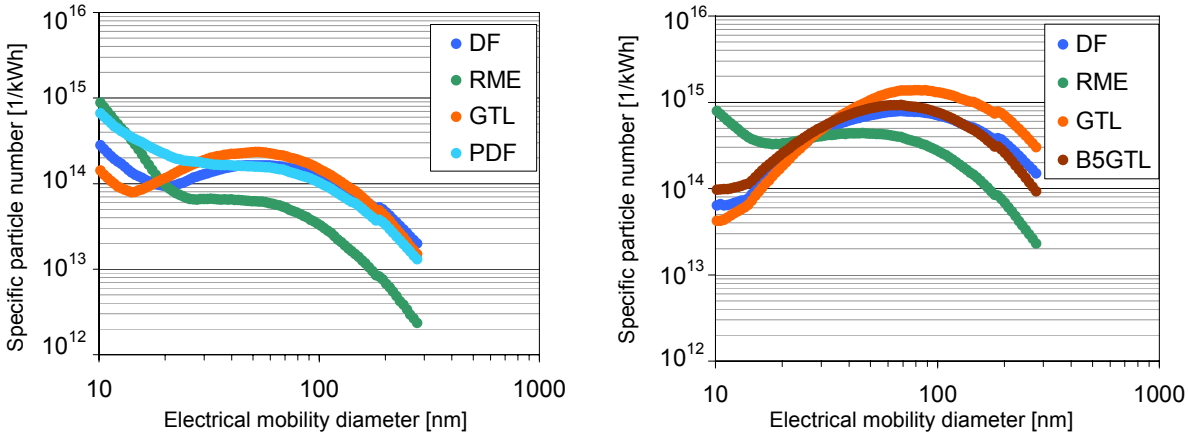


Figure 6 Particle size distributions (ELPI) of different fuels, ESC test, OM 906 LA; two separate measurement campaigns

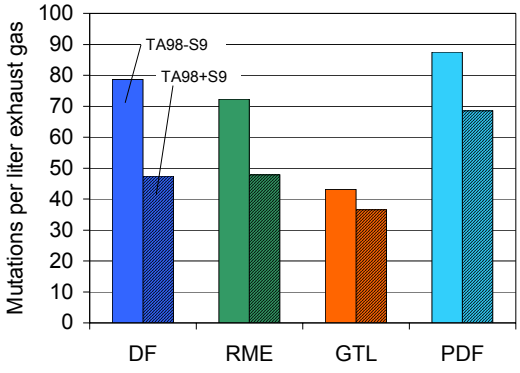


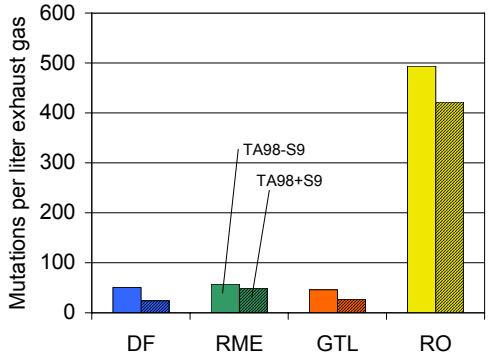
Figures 7, 8. Particle size distributions (SMPS) of different fuels, ESC test, OM 906 LA engine; two separate measurement campaigns

Measurement of electrical mobility diameters via SMPS allows determination of ultra fine particles (UFP) as small as 10 nm (Figs. 7, 8). In this range RME emitted more particles than DF and GTL, while for larger particles the opposite was the case. PDF showed overall higher emissions than would be suggested from its composition in case of linear effects. With B5GTL, however, a linear behaviour was observed.

Results of **mutagenicity** testing are displayed in Figs. 9 and 10. Mutagenicity of exhaust gases was found to be almost the same when DF and RME were used as engine fuel. GTL showed less mutagenic potency; B5GTL had not been tested in this study. As an important result, the three-component blend PDF caused higher mutagenicity than each of its components. It is not yet clear, which type of synergistic effects may be responsible for this phenomenon.

In a second test series, GTL, RME und DF were compared to RO. It could be demonstrated that the mutagenic activity of the latter was up to tenfold higher than that of RME and DF. Here, the vegetable oil had significant disadvantages. This means that the mutagenicity of this exhaust gas lay in the range of diesel engine emissions of the 1980s [6].





Figs. 9, 10. Mutagenic potency of different fuels, ESC test, OM 906 LA; two measurement series

This result concerning mutagenicity finds its parallel in a former result, obtained by Ricardo Consulting Engineers, on increase of PAH emissions when using plant oil as diesel fuel in a DI diesel engine [7]. The authors reported 20-fold higher emissions of Benzo(a)pyrene for vegetable oil, compared to conventional diesel fuel.

Further and systematic research into this direction is urgently needed and strongly recommended!

Acknowledgement

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References

[1] Krahl, J., Munack, A., Schröder, O., Stein, H., Herbst, L., Kaufmann, A., Bünger, J.: Fuel Design as Constructional Element with the Example of Biogenic and Fossil Diesel Fuels. Agricultural Engineering International. Vol. VII, Manuscript EE 04 008, http://cigr-ejournal.tamu.edu (2005).

[2] Ames, B.N., McCann J., Yamasaki E.: Methods for detecting carcinogens and mutagens with the Salmonella /mammalian-microsome mutagenicity test. *Mutation Res*. 31 (1975), 347-363.

[3] Maron, D.M., Ames B.N.: Revised methods for the Salmonella mutagenicity test. *Mutation Res*., 113 (1983), 173-215.

[4] Matsushima, T., M. Sawamura, K. Hara, and T. Sugimura: A safe substitute for polychlorinated biphenyls as an inducer of metabolic activation system. In: In Vitro Metabolic Activation in Mutagenesis Testing, ed. F.J. Serres, J.R. Fouts, R.M. Bend, and R.N. Philpot. North-Holland, Amsterdam: Elsevier 1976, 85-88.

[5] Krewski, D., Leroux, B.G., Creason, J., and Claxton, L.: Sources of variation in the mutagenic potency of complex chemical mixtures based on the Salmonella/microsome assay. *Mutation Res*. 276 (1992), 33-59.

[6] Greim, H., Hillesheim, W., Esch, H., Höke, H., Zwirner-Baier, I.: Vergleich der Wirkungseigenschaften und der Wirkungsstärke von Dieselmotorabgasen der 1960er Jahre und heute. Abschlussbericht. Institut für Toxikologie und Umwelthygiene, Technische Universität München, 2003.

[7] Lance, D.L., Andersson, J.D.: Emissions performance of pure vegetable oil in two European light duty vehicles, SAE-Paper 2004-01-1881, 2004.

[8] Munack, A., Herbst, L., Kaufmann, A., Ruschel, Y., Schröder, O., Krahl, J., Bünger, J.: Vergleich von Shell Mittel-Destillat, Premium-Dieselkraftstoff und fossilem Dieselkraftstoff mit Rapsölmethylester. Abschlussbericht. Institut fürTechnologie und Biosystemtechnik, Bundesforschungsanstalt für Landwirtschaft (FAL), 44 S., Braunschweig, 2005.

[9] Munack, A., Herbst, L., Kaufmann, A., Ruschel, Y., Schröder, O., Krahl, J., Bünger, J.: Comparison of Shell Middle Distillate, Premium Diesel Fuel and Fossil Diesel Fuel with Rapeseed Oil Methyl Ester. Final Report. Institute for Technology and Biosystems Engineering, Federal Agricultural Research Centre (FAL), 41 p, Braunschweig, 2005.

Biodiesel in Germany - market trends and competition

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Abstract

According to experts' opinion, for now, only biofuels as an alternative to fossil fuels will be able to make a noteworthy contribution to secure mobility in the short to medium term. This was emphasised by the Association of the German Automobile Industry (VDA) in February 2006 when the VDA self-imposed the obligation for the car industry to lay the technical foundations for a 10 % admixture of biofuels to diesel fuel and gasoline.

The European Commission In January 2007 the proposals for a "road map for renewable energies" with the aim to make more pressure on the member states of the enlarged European Union – EU27. So the challenge is to open the market to biofuels and at the same time to push the optimisation of combustion engines in order to reduce fuel consumption and emissions.

Key words: biodiesel, biofuel quota act, DIN EN 14214, taxation, biodiesel market

Introduction

The main factors for the political motivation to promote the use of biofuels are the protection of the climate, securing the fuel supply, and the development of rural areas. Given the rapid rising of the crude oil price and increasing geopolitical uncertainties, the security of energy supply does not only govern politics in Germany but around the globe.

As an element of the national and European energy supply, the German and European biomass potential is gaining more increasing importance. This is supposed by the "EU Biomass Action Plan" suggested by the EU Commission at the end of 2005 and the EU strategy for biofuels from January 2006. In January 2007 the EU-Commission published the "Renwable Energy Road Map" with the proposals for the new targets for a further increase of the consumption renewable energy up to 20% until 2020. Especially for biofuels should be introduced a mandatory target: 10% of the fuel consumption on energy basis. It is imperative to do as much as possible in the given timescale as agricultural industry expectations will be extraordinarily high when Germany has taken over the presidency of the Council of the EU in January 2007.

German parliament resolves partial taxation

By passing the amendment to the Mineral Oil Tax Act on January 1, 2004 to grant tax privileges for biofuels, Germany created legally binding regulations for biofuels. On the one hand the adoption of the EU Directive (2003/30/EG) on the Promotion of Biofuels, as well as the Energy Taxation Directive (2003/96/EG), restructuring the framework for the taxation of energy products, provided the pressure and urgency to promote biofuels as a strategic element of fuel supply. Those were based on specified EU targets. On the other hand authorisation was given to reduce the taxation of biofuels on national level for Member States. In February 2004 the EU Commission granted Germany's application to exempt biofuels from mineral oil tax from January 1, 2004 until December 31, 2009. As a result of this notification the EU Commission acknowledged that not only production costs have to be taken into account in order to determine the extent of any tax relief. The lower energy content of biofuels (use of pure fuels), resulting in an increased fuel consumption as an additional "cost increasing factor" must also be considered. This „cost increasing factor" does not apply to the mineral oil industry for admixtures to Diesel fuel (max. 5 % of biodiesel according to DIN EN 590) or gasoline (max. 5 % of bioethanol according to DIN EN 228).

As far as biodiesel and bioethanol were concerned, the Commission concluded that the national mineral oil tax exemption will not lead to any overcompensation. The Commission approved the measures defined in the German Mineral Oil Tax Act concerning an annual review of any overcompensation by means of a report to the German Bundestag (over-compensation-check). The Commission stated that any financial aid is to be restricted to the compensation of the competition disadvantage due to higher production costs of biofuels in comparison to Diesel fuels. In 2004 and especially in 2005 this situation changed due to rising crude oil prices. In its coalition agreement from November 2005 the new German Coalition Government emphasised the particular importance of biomass as a source of renewable energy. However, with reference to the anticipated tax revenue shortfall due to the rapidly growing biodiesel and bioethanol capacities, the consolidation of the Federal Government Budget came to the fore. The coalition negotiations initially aimed at completely replacing tax privileges by an obligation to blend biofuels. This intention was not only opposed by industrial associations of the biofuel industry, UFOP and the German Farmers' Association ("Deutscher Bauernverband") but also by the automobile industry.

UFOP - as well as other associations – had intensive talks with delegates of the Coalition Government about the possible consequences for the biodiesel and agricultural industries, should the Energy Tax Act with its initially planned tax rates enter into force in 2009 after the granted tax relief period had expired. Finally, the Coalition Government not only accepted the protection of confidence concerning any made investments and production plants currently under construction but also the necessity that a reliable framework needs to be created beyond 2009. In principal the German Federal Government will follow

the strategy to combine tax privileges and administration law in order to live up to the constraints regarding the consolidation of the Federal Budget as well as to meet the target quantities of the action plan of the European Union.

The Biofuel Quota Act

On 1st of January 2007 the Biofuel Quota Act went into force. As a consequence of that the following gradual reduction of tax privileges for biodiesel and vegetable oil took effect:

Taxation of Biofuels

Year	tax rates	tax rates
	biodiesel	vegetable oil
2007	9 Cents/litre	0 Cent/litre
2008	15 Cents/litre	10 Cents/litre
2009	21 Cents/litre	18 Cents/litre
2010	27 Cents/litre	26 Cents/litre
2011	33 Cents/litre	33 Cents/litre
from 2012	45 Cents/litre	45 Cents/litre

Principally, UFOP welcomes the fact that the German Federal Government followed the claim of UFOP and the DBV Deutscher Bauernverband (German Farmers' Association) to exempt agriculture and forestry permanently from partial taxation without any quotas.

At present the national tax regulations of the individual EU Member States for Diesel fuel taxation used in agriculture differ considerably. But the tax rates for the pure fuel market (forwarding trade, public filling stations) are considered as too high. There is a considerable risk involved that the commercialisation of pure biodiesel and vegetable oil may already become unprofitable in 2008/09.

UFOP demands that the amendment has to take into account that the guideline for stipulating the tax rate for biodiesel and vegetable oil fuels has to guarantee the competitiveness of biofuels and their analogue fossil fuels. Differing energy contents and additional technical expenditure necessary for the operation with the specific biofuel (biodiesel, vegetable oil, E85) must be part of any assessment. It is likely that already by 2008, the market for pure biofuels might be at a considerable competitive disadvantage, should the price for Diesel fuel possibly drop.

The Regulations at a glance

Precondition for tax privilege for:

- Biodiesel – DIN EN 14214
- Vegetable Oil – DIN V 51605

Biodiesel generated from animal fats for pure fuel use:

tax privileges are limited until December 31, 2011

No taxation until 2015 for:

- Synthetic biofuels (biomass to liquid – btl)
- Bioethanol based on hemicellulose
- E85 (blend of 85 % bioethanol, 15 % gasoline)

- Subject to an annual revision regarding overcompensation

- Consensus essential from the Ministries of Agriculture, Environment, Traffic and Finance concerning minimum criteria for a sustainable farming of agricultural areas or/and the conditions to save carbon dioxide/CO_2.

Amendment of the Federal Immission Control Act

Introduction of biofuel quotas (bonded warehouses/free circulation) for:

- Producers and traders of Diesel fuel and gasoline
- Producers of biofuels (biodiesel, vegetable oils)

Biodiesel/ Bioethanol Quota Obligation

Year	Total quota	Diesel quota	Petrol quota
2007	-	4.40%	1.20%
2008	-		2.00%
2009	6.25%	Minimum quota	2.80%
2010	6.75%	applies also to	3.60%
2011	7.00%	subsequent years	
2012	7.25%		Minimum quota
2013	7.50%		applies also to
2014	7.75%		subsequent years
2015	8.00%		

* Proportion of the entire energy necessary for transport purposes

Preconditions for Tax Privileges and Eligibility concerning Quota Obligation

Biodiesel – DIN EN 14214
Plant oil – DIN V 51606
Bioethanol – 99 vol % alcohol content

Vegetable oils used directly in the refining process are not eligible.

New/innovative technologies are accepted, e.g. hydrogenation of vegetable oils according to the „Neste-Oil-Process" („NextBTL).

Regulation of sanctions in case of non-compliance (based on caloric value):

- Diesel - biodiesel – 16 Euros per GJ (50 Cents/litre)
- Gasoline - bioethanol – 28 Euros per GJ (80 Cents/litre)
- Additional overall quota - 16 Euros/GJ (50 Cents/litre)

UFOP welcomes that the granting of tax privileges is dependent on the fulfilment of the applicable standards for biodiesel – DIN EN 14214 – and vegetable oil fuel – DIN V 51605. Up to now biodiesel could be imported from EU and non EU countries virtually without any quality control. Sometimes this led to severe problems for the final user. Often producers and retailers are confronted with the impairment of their image despite efforts of assuring quality production for their (final) customers and their endeavour to improve the quality beyond the requirements of the current standards. It is mandatory that biodiesel producers keep up with the general development concerning the quality of fossil Diesel and the improved engine and exhaust gas after-treatment technologies. The German Federal Government signals further that,

1. apart from rapeseed oil other feedstocks can be used to a limited extent if the requirements according to the standard are fulfilled;

2. excessive imports – which the public would find difficult to accept – of raw materials and/or biodiesel from countries outside the EU will be prevented.

The biodiesel industry is now well established in the public opinion due to a high level of transparency which comprises the production of raw materials and biodiesel itself. This also applies to a multitude of small companies – including agricultural ones – which are involved in the commercialisation of pure fuels. On the other hand the utilisation of palm and soy oils as a raw material for the production of biodiesel encounters public resistance. It raises concern that tax privileges and political incentives in Germany or within the EU may result in the promotion of the soya bean cultivation in Northern and Southern America or provoke primeval forest clearings for the creation of palm oil plantations.The demanded creation of a certification system for the production of raw materials and/or the required criteria for sustainable operation must now be coordinated at EU level in order to rule out any commercial conflicts right from the start.

Production of biodiesel

By the end of 2007 the production capacity of biodiesel will have reached almost 3,4 to 3,7 mio. tonnes. Compared with 265.000 tonnes in the year 2000 the capacaty for the production of biodiesel has increased very fast. Based on the national production capacity, biodiesel could already cover about 12 % of the consumption of Diesel fuel by 2008. Hence mandatory blending quotas are an important "safety net" for any future biodiesel sale. However, only about 1.3 to 1.4 million tonnes of biodiesel can be sold to the mineral oil industry if 5 vol % are admixed to diesel fuel according to the European Diesel fuel Standard EN 590.

Therefore, in view of the rapidly growing capacities, the Federal Government and the European Commission must see to the fact that the European standard for diesel fuels will allow a 10 vol % admixture of Biodiesel. The EU Commission – also upon request of the German Government – supplied a draft of a mandate to the European Committee for Standardization (CEN) for the consideration of a revision of the standard as soon as possible in consensus with the industry concerned.

After all, in recent years about EUR 400 to EUR 500 million were invested into the build-up of the biodiesel production. Given the taxation of biodiesel according to the Energy Tax Act 2007, there is a legitimate fear that overcapacities cannot be ruled out and the pressure to export biodiesel will increase. However, by no means is there an existing internal EU market for biofuels – on the contrary, some countries, especially France, implement a policy of impeding imports. As a result of French tendering procedures virtually no biofuel producers other than French ones can do business in France. In contrast, French enterprises can export biodiesel to Germany at all times and without any quantity restrictions.

Today Germany is not only the world's leading producer of biodiesel but as a result also ahead with regard to the development of production plant technology. The large increase of enquiries from abroad, concerning visits to production facilities, confirms the growing international interest in German plant engineering and construction technology which is turning into a grand success.

The sustainable supply with rapeseed oil as raw material is a crucial strategic element to ensure the competitiveness of a biodiesel production plant. Especially in years when due to weather conditions the crop does not live up to expectations, high vegetable oil prices and/or the missing availability of rapeseed oil could threat the existence of the production plants. Therefore, the plant's location and effective access to the infrastructure, especially to navigable rivers and canals, is vital for its competitiveness. As a consequence of the increasing demand for rapeseed oil, the crushing capacity of oil mills will increase from 6 million tonnes at present to 7.5 million tonnes by the end of 2007. Existing oil mills will either be enlarged or integrated into biodiesel production plants or built in direct proximity.

Establishment of biodiesel on the market

Parallel to the capacity development, biodiesel sales achieved again an all-time peak value of all in all 1.8 mio.tonnes in the year 2005 and estimated 2,2 mio. tonnes 2006. In 2005 about 1.5 million tonnes of biodiesel were sourced from domestic production, while already 300,000 to 400,000 tonnes were imported. Reliable statistical data is not available so far, due to still missing integration of biofuel data in the relevant mineral oil statistics. Nonetheless, biofuels are subject to the same obligation to report as fossil fuels. So there is still a need for the creation of a reporting network which is under discussion at present amongst the responsible authorities (Federal Ministry of Finance, Federal Ministry for Economy, Federal Ministry for the Environment and Federal Ministry for Agriculture) and the biofuel industry.

According to an assessment carried out by (AGQM *Arbeitsgemeinschaft Qualitätsmanagement Biodiesel e.V. / Association for the Quality Management of Biodiesel e.V – www.agqm-biodiesel.de.*) (January 2006), filling station operators invested some 8 million Euros for the construction and conversion of public biodiesel filling stations in 2005 alone, totalling about 35 million Euros since 1996. For those companies, with a sold quantity of about 520,000 tonnes (about 420,000 tonnes in 2004), biodiesel has turned into an important additional source of income in a fiercely competitive fuel market. In 2005 a total of 1 million tonnes of biodiesel were sold as pure fuel directly to large-scale consumers (forwarding trade and public transport companies) and for private use at public filling stations. Especially imports have been and remain often of inadequate quality which resulted in complaints during autumn and winter of 2005. For that reason UFOP recommend to exclusively purchase biodiesel of assured quality of AGQM members.

Meanwhile, the network of public biodiesel filling stations comprises about 1,900 stations – about one in nine public filling stations in Germany offers biodiesel. 1.400 of those 1.900 biodiesel filling stations are associated with the quality management system of AGQM. The market segment of passenger cars will gradually lose importance due to the lack of approvals for the operation with biodiesel. The reason is the decision of the member companies of the Volkswagen Group (VW, Audi, SEAT, Skoda) to offer EURO-4-engines in combination with self-regenerating particle filter systems, to account for the discussion on particulate matter. In order to secure the existing potential of custom in the car sector, UFOP support two projects on the assessment of diesel particle filter systems for retro-fitting in old cars. These projects had been successful completed in autumn 2006.

The receding sale of biodiesel at filling stations will result in a regional decline of the number of filling stations. The removal of biodiesel from the range of products at the filling stations is to be recommended, should the turnover decline. Otherwise, as a consequence of the prolonged storage period, problems could arise in case of unannounced official quality control checks by the authorities. AGQM's checks at public filling stations have already led to the cancellation of brand licence contracts. This affects primarily filling stations which do not have any facilities to serve commercial vehicles (time of fuel turnover). Therefore, biodiesel in its pure form will be increasingly sold to the forwarding trade which will lead to more competition concerning that particular clientele. For that reason, only filling stations in urban areas or near motorways might add biodiesel to their range of products – usually instead of premium gazoline "Super plus" – thus compensating for some of the decrease in the number of biodiesel filling stations.

The petroleum industry admixed about 600,000 tonnes of biodiesel to Diesel fuel in proportions of up to 5 % vol. according to DIN EN 590. According to information from the MWV Mineralölwirtschaftsverband (*Association of the German Petroleum Industry*) 1,000,000 tonnes of biodiesel could have been admixed which failed for lack of biodiesel availability in 2005.

Releases secure sales

The commercial vehicle sector is the „biodiesel customer" showing the strongest demand for biodiesel by far. DaimlerChrysler, MAN and IVECO gave approvals for EURO-4 and EURO-5 truck engines in commercial vehicles. Moreover, DaimlerChrysler is offering extra equipment (bigger oil sump, separate fuel supply for the auxiliary heating system) to enable the utilisation of biodiesel. Various factors contributed to this marketing policy. One of the main reasons was the pressure from customers of the forwarding trade. Another important reason was the fact that quality control measures by AGQM showed considerable improvement of the biodiesel quality, especially in terms of the content of the so-called 'ash forming substances' (Calcium, Magnesium and others). That way any misgivings by the manufacturers of commercial vehicles with regard to the compatibility of modern exhaust gas after-treatment devices (SCR, BlueTec) and biodiesel were disproved. Concerning the requirements of modern Diesel engines, all prerequisites are already in existence for biodiesel to remain the most important fuel alternative in the medium-term, mainly as pure fuel for the operation of commercial vehicles.

Dependent on the development of the price for fossil diesel fuel, the agricultural and forestry sectors will gain considerably in importance concerning the commercialisation of pure biofuels following lasting tax exemption. The annual consumption of this commercial sector amounts to about 1.5 mio. tonnes of fossil diesel. The market potential for biodiesel is currently estimated to at around 300,000 to 400,000 tonnes considering the regulation for tax refunds of agricultural Diesel fuel (limited to 10,000 litres/farm). Apart from few exceptions, virtually all engines are approved for biodiesel. In 2006 UFOP successfully supported a project by DEUTZ AG with the aim to achieve approval for the use of biodiesel for engines of the latest generation which already comply with the regulations of emission level Tier III B. Therefore, it will also be possible in the agricultural sector to use biodiesel as pure fuel in the future.

Rapeseed oil fuel

Notable investments estimated at 60 to 70 million Euros were also made in small pressing facilities for obtaining

rapeseed oil. During the past three years the number of decentral pressing facilities rose sharply from 98 to more than 300 with a grinding capacity of 0.5 to 0.6 million tonnes. Mainly rapeseed oil is produced for the use as pure fuel or as raw material for the production of biodiesel. UFOP assumes that their cooperation with biodiesel producers will be intensified as a result of permanent tax exemption for the agriculture. In July 2006 the pre-standard for rapeseed oil fuel – DIN V 51605 – was presented. From the very beginning UFOP financially supported this standardisation project with the purpose to ascertain the definition of terms according to the specified quality parameters, and thus the legal relationship between the end user and the fuel producers and/or traders. In addition to tax regulations, the demand pressure for high quality will be increased to offer the best possible protection for the consumer. Especially rapeseed oil producers will have to deal with this challenge. However, all participants are aware of the fact, that the standardisation process is not completed by the creation of a pre-standard. Rapeseed oil fuel according to DIN V 51605 has to be the quality on which comparable exhaust emission trials are to be based. These tests were not included in the 100-tractor-programme of the federal agency for renewable resources (www.fnr.de).

It is already clear that the limits for the ash-forming components (Calcium, Magnesium and others) as well as for phosphorus (deactivating agent for catalytic converters) will have to be subject to further reduction.The Bavarian Technology Center for Renewable Ressources (TFZ) anticipated that small-scale facilities can still comply with more stringent requirements. However, they will experience a lower oil yield. From UFOP's point of view, the question for efficiency reasons arises if the crushing grade should be elevated. In order to be able to reliably guarantee the parameters for fuel quality, the regional erection of oil refining plants should be taken into consideration. This necessity can be derived from the results of the 100-tractor-programme in which, for a period of three years, 107 retrofitted tractors were tested in a large-scale field test with regard to their environmental soundness, endurance and field performance when operated with rapeseed oil. 63 of the 107 tractors (60 %) completed the project period without any or with very few disruptions. Some technical deficits concerning the engines and the quality of the fuel were identified and require further attention so that mass production maturity and practical performance capability can be achieved.

UFOP, the Schleswig-Holstein-Foundation and FNR cooperate in funding a project for converted tractors which did not take part in the 100-tractor-programme. Again, the experiences with retrofitting systems suitable or unsuitable for the operation with rapeseed oil are confirmed. The results are used during training lessons for farmers as well as the training and further education of mechanics for agricultural machinery.

Summary

With its strategy for biofuels and its road map, the EU Commission intends to press ahead with the market introduction of biofuels all over the EU. This is motivated by the substantial and otherwise rising dependency on imports, especially of crude oil, as well as the possible contribution of biofuels to securing mobility. The EU Commission, the automotive industry and the agriculture sector agree on that only biofuels can contribute significantly in the near future. The Member States of the EU will be urged to favour biofuels by taking into account the fast increasing raw material demand. In addition obligatory use of biofuels is to be revised. The possible committment of 10 % market share combined with the promotion of energy from biomass for heat and electricity supply will be a challange and a chance for the agriculture to develop new fields for enterpreneurs.

References

Commission of the European Communities

Renewable Energy Road Map – Renewable energies in the 21st century: building a more sustainable future, 24.11.2006

Biofuels Progress Report – Report on the progress made in the use of biofuels an d other renewable fuels in the Member States of the European Union, 09.01.2007 COM(2006) 845 final

Commission Staff Working Document – Review of economic and environmental data for the biofuels progress report (27.11.2006) draft version 2

Transesterification of vegetable oilwith potassium cabonate/active carbon catalyst

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Abstract

It is a hot topic of producing biodiesel with heterogeneous catalyst nowadays. Transesterification of oil with methanol using potassium carbonate/active carbon as catalyst, and the effects of catalyst dosage, methanol proportion, reaction time, etc., on the reaction were studied. The results showed that potassium carbonate/active carbon had strong catalysis for the transesterification of oil with methanol; potassium carbonate/active carbon could be reused by recovery. The transesterification rate could be above 98% by two-step transesterification.

Key words: grease; transesterification; solid catalyst; potassium carbonate/active carbon

1. Introduction

Transesterification with renewable animal and vegetable oils can attain mixture of fatty acid methyl esters and ethanol esters. The mixture is called biodiesel. Conventional synthesis techniques of biodiesel rely on homogeneous acid/base catalysts (Edgar et al., 2005). However, in the conventional homogeneous manner, removal of these catalysts is technically difficult and a large amount of wastewater is produced to separate and clean the catalyst and the product. To avoid the problem of products separation, it has been proposed to replace the homogeneous catalysts by a heterogeneous catalyst, suggesting that the production of alkyl esters will be simplified when heterogeneous catalysts are utilized.

Many different heterogeneous catalysts have been developed to catalyze the transesterification of vegetable oil to prepare fatty acid methyl esters. For example, CsX zeolites, anionic clays, calcium carbonate rock, EST-10, Li/CaO and Na/NaOH/γ-Al2O3 have been found to be efficient heterogeneous catalysts for the transesterification of vegetable oil (Kim et al.,2004). However, they are quite expensive or complicated to prepare, which limits their industrial application.

In this work, the potassium carbonate/active carbon catalyst was adopted for the production of biodiesel from rapeseed oil.

2. Experimental

2.1 Catalyst preparation

Active carbon, obtained from NanKai Gongyun Co. (Tianjin, China), was dried in 221 K for 1.5 h to remove the absorbed water on the surface. To prepare modified Potassium carbonate/active carbon catalysts with different K2CO3 loadings, the active carbon was impregnated with an aqueous solution of K2CO3. Samples with various K2CO3 loadings, given in weight percentage, were impregnated thoroughly on the surface of active carbon. The pretreated impregnated samples were dried in 221K and then heated in an oven at 932K for 5 h before use for the reaction. The loading amounts of K2CO3 were calculated on the basis of the amounts of the starting materials.

2.2 Transesterification procedure

Commercial edible grade oil was obtained from market and was refined again to reduce free fatty acid and water contents further. The transesterification reaction was carried out using different methanol/oil molar ratios and various amounts of catalyst. The mixture was stirred and refluxed for the required reaction time. After the reaction time finished, the mixture was filtered and the residual methanol was separated from the liquid phase via rotary evaporation prior to analysis.

For GC analysis, the reaction mixture, after methanol was removed completely, was washed three times with a saturated aqueous NaCl solution for removal of the formed glycerin. The separated organic phase was dried with anhydrous MgSO4, and then submitted to GC analysis (Angilent, GC 6890). The conversion efficiency of the edible oil to methyl esters was determined by the content of the methyl esters.

3 Results and discussion

3.1 Influence of loading amount of K2CO3 on the conversion

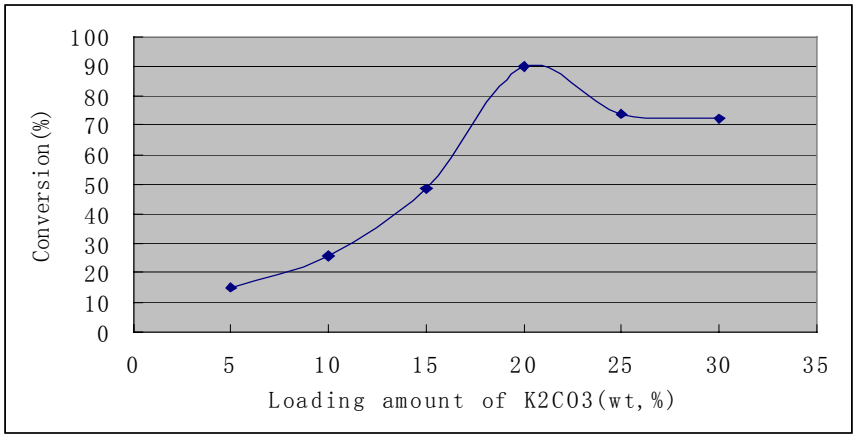

Fig.1. Influence of loading amount of K_2CO_3 on the conversion.

Reaction conditions: methanol/oil molar ratio 8:1, catalyst amount 5 wt.%, reaction time 5 h and reaction temperature 158 K.

The dependence of the activity of K_2CO_3/C catalysts on the loading amount of K_2CO_3 was investigated. The results are shown in Fig. 1. As expected, the active carbon did not present any particular catalytic activity, most likely due to the lack of strong basic sites on which the transesterification reaction could occur. However, loading of K_2CO_3 onto the active carbon produced a dramatic increment of basic strengths on the K_2CO_3/C catalyst as mentioned previously, and due to this, resulting in an increase in the conversion to methyl ester (Fig.1.). Thus, the production of biodiesel in the reaction of vegetable oil with methanol needed strong basic sites. As shown in Fig. 1, when the loading amount of K_2CO_3 increased, the conversion increased and the highest conversion of 85.6% was registered at loading of 20%. However, when the loaded K_2CO_3 was over 20%, the conversion decreased. It was very likely that the amount of loaded K_2CO_3 higher than 20% resulted in the agglomeration of the active K_2CO_3 phase or the cover of basic sites by the exceeded K_2CO_3, hence lower the surface areas of active components and lower the catalytic activity.

3.2 Influence of methanol/oil molar ratios on the conversion

Fig. 2 shows the effect of methanol/oil molar ratios on the conversion. By increasing the amount of loading methanol, the conversion of vegetable oil increased considerably. When the methanol/oil molar ratio was very close to 6:1, the conversion reached the maximum value of 91.56%. However, beyond the molar ratio of 8:1, the excessively added methanol had no significant effect on the conversion. Therefore, the optimum molar ratio of methanol/oil to produce methyl esters was approximately 8:1. However, it has been found that the methanol/oil molar ratio of 6:1 could give the best conversion when the transesterification reaction was carried out using conversional homogeneous catalysts such as NaOH and KOH. Generally, homogeneous catalysts have higher activities than heterogeneous catalysts. However, recent studies on the transesterification of vegetable oil with methanol in the presence of ETS-10 zeolite, and metal catalysts (Suppes et al., 2004) showed that heterogeneous catalysts containing occluded reactor gave >90% conversion at 393 K in 24 h with 6:1 molar ratio of the oil to methanol, and that ZnO gave the highest conversion of 80% at 393 K in 24 h among the metal catalysts.

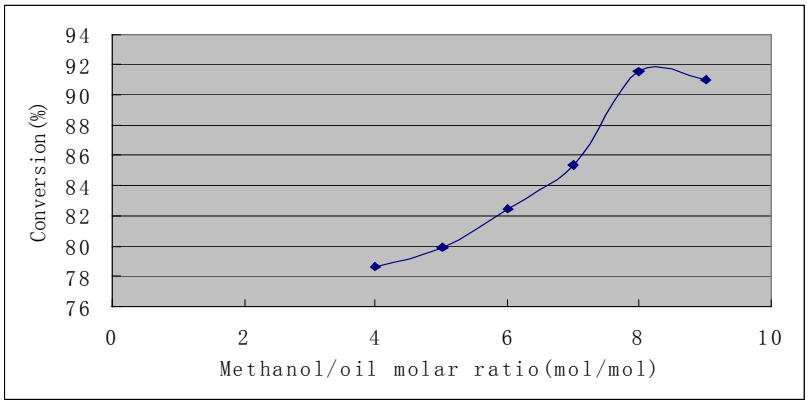

Fig. 2. Influence of methanol/oil molar ratios on the conversion.

Reaction conditions: catalyst amount 5 wt.%, reaction time 5 h and reaction temperature 158 K.

3.3 Influence of catalyst amount on the conversion

The effect of the catalyst amount was studied. The catalyst amount was varied in the range of between 1.0% and 6.0%,

the percent was weight ratio of catalyst/starting oil. Fig 3 showed that the transesterification reaction was strongly dependent upon the amount of catalyst applied. The transesterification procedure did not occur without addition of a catalyst, and the presence of the K_2CO_3/C catalyst really increased the reaction rate. Increased catalyst amount from 1% to 5% resulted increased conversion of methyl esters (from 37.65% to 89.46%). However, with the increasing in the catalyst amount, the conversion was decreased, which was possibly due to the rise of a mixing problem of reactants, products and solid catalyst.

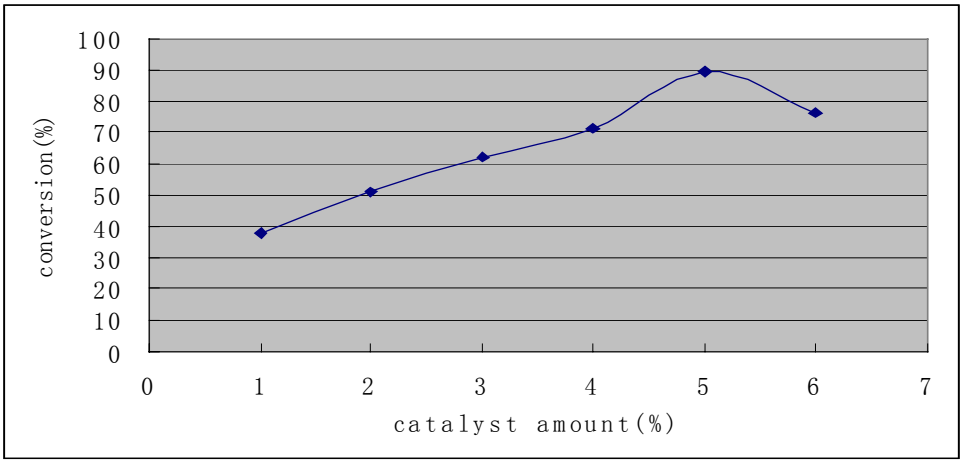

Fig. 3. Influence of catalyst amount on the conversion

Reaction conditions: methanol/oil molar ratio 8:1, reaction time 5 h and reaction temperature 158 K.

In order to study the stability of K_2CO_3/C catalyst, the separated catalyst was reused without activation treatment, the conversion was 62.01%

3.4 Influence of reaction time on the conversion

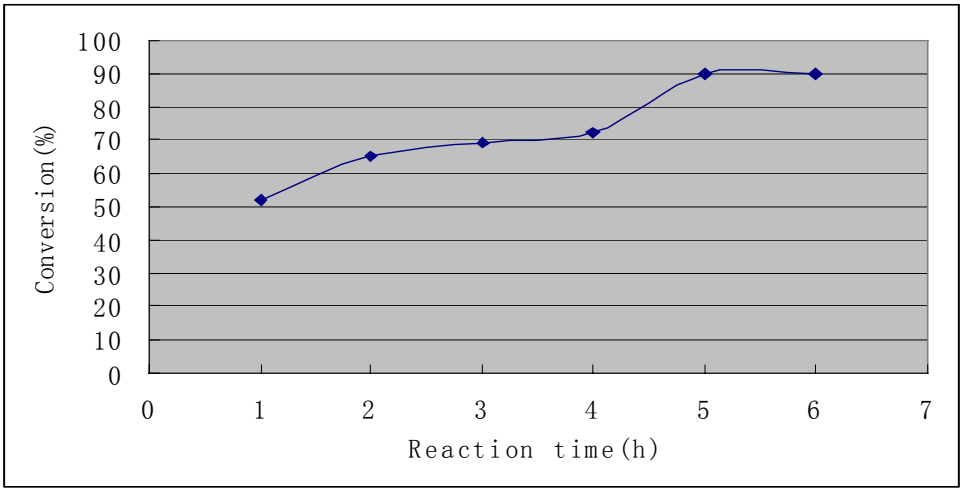

Fig.4. Influence of reaction time on the conversion.

Reaction conditions: methanol/oil molar ratio 8:1, catalyst amount 5 wt.% and reaction temperature 158 K.

Fig 2 illustrates the change of the conversion as a function of reaction time. The optimum reaction time for the production of biodiesel was determined by performing reactions at varying reaction time. For the same reaction conditions, the conversion increased in the reaction time range from 1 h to 6 h, when the reaction time reached 5 h, the conversion rate was the best (90.13%). Therefore, 5 h reflux of methanol was considered as optimum reaction time.

The effect of mixing played an important part in the transesterification reaction. The reaction was carried out at two randomly chosen stirring speeds (300rpm and 600rpm). At lower stirring speed, the oil conversion reached 58.6% after 5 h of reaction, whereas at 600 rpm the oil conversions. The result showed that an efficient mixing of the reagents was essential to reach a high conversion of the oil.

3.5 Influence of stability of catalyst on the conversion

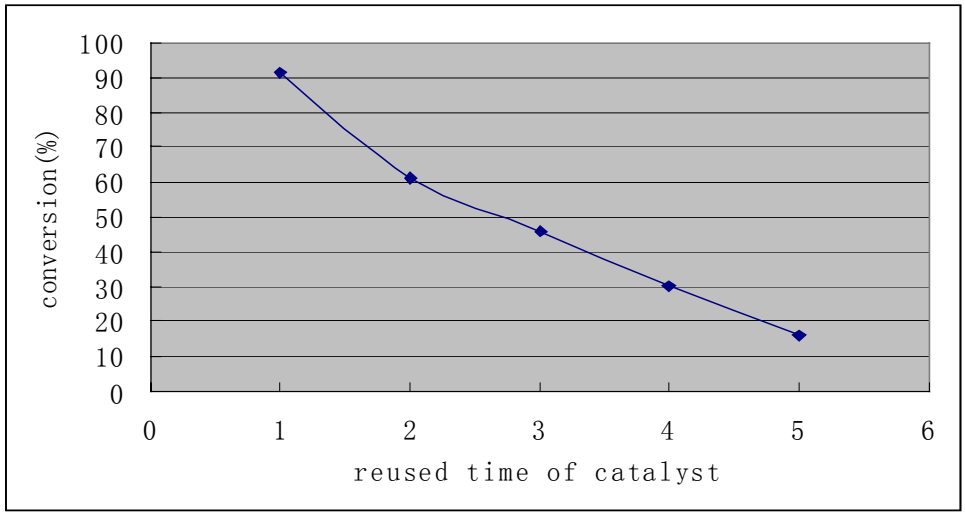

Fig.5. Influence of stability of catalyst on the conversion.

Reaction conditions: catalyst amount 5%, reaction time 5 h, reaction temperature 158 k and methanol/oil molar ratio 6:1.

In order to study the stability of K_2CO_3/C catalyst, it was reused by separating the catalyst without activation treatment. The result showed that the conversion decreased greatly after the first transesterification reaction. Hence, the solid catalyst must be activated after it was used. The treatment process is generally that washing it thoroughly with organic impregnant and heating in an oven for 2 h at 398 k.

4. Conclusion

The loading of K_2CO_3 on the active carbon resulted in an increase in the basic strength over the active carbon, and the supported catalyst can easily be separated and reused time after time. After being loaded with K_2CO_3, the pore structure of active carbon, necessary for catalysis could be retained. This approach was successfully used in an attempt to increase the catalytic activity of solid catalyst towards the transesterification reaction.

References

Edgar, L., Liu, Y., Lopez, DE., Suwannakam, K., Bruce, D.A., Goodwin, J.G.., 2005. Synthesis of biodiesel via acid catalysis. Ind. Eng. Chem. Res, 44, 5353-5363.

G.J.Suppes.et a1.Transesterification of Soybean oil with zeolite and metal catalysts [J].Applied catalysis A:General, 2004, 257:2 l3—–223.

Kim, H.J., Kang, B.S., Kim, M.J., Park, Y.M., Kim, D.K., Lee, J.S., Lee, K.Y., 2004, transesterification of vegetable oil to biodiesel using heterogeneous base catalyst. Catal. TOoday 93-95, 315-320.

A.Corma,S.Iborra,S.Miquel,J.Primo.Catalysts for the production of fin chemicals[J]. Journal of catalysis,1998,173,315-321.

Gryglewicz S.Rapeseed Oil Methyl Esters Preparation Using Heterogeneous Catalysts.Bioresour Technol, 1999, 70(3):249~253.

Emissions and health effects from heavy-duty engines running on alternative fuels

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Abstract

Two batches of rapeseed oil methyl esters containing approximately 10 ppm phosphorus (RME_{10}), one rapeseed oil methyl ester with a content of less than 1 ppm phosphorus (RME) and common diesel fuel (DF) were investigated regarding their effects on regulated and non-regulated emissions at a modern diesel engine (Euro IV), equipped with an SCR system (selective catalytical reduction of nitrogen oxides). The regulated emissions of carbon monoxide (CO), hydrocarbons (HC), nitrogen oxides (NO_x) and particulate matter (PM) were determined for RME_{10} and DF. Non-regulated emissions of alkenes, alkynes, aromatics, aldehydes, ketones and the particle size distribution were measured for all fuels. Additionally the mutagenic potency of the particulate matter was determined by Ames tests.

RME_{10} led to lower regulated emissions than conventional diesel fuel. Regarding the non-regulated emissions RME showed the lowest values compared with RME_{10} and DF. The catalytic activity of the SCR system was reduced after an endurance test of 1000 hours using RME_{10} as fuel resulting in higher regulated and non-regulated emissions.

The mutagenicity of all PM extracts was very low compared to prior studies. This is probably due to an effective conversion by the SCR system. Nevertheless, mutagenic effects of PM extracts increased moderately after the endurance test.

Key words: Diesel engine; exhaust gas emissions; SCR system; RME; phosphorus content; mutagenicity.

Introduction

One characteristic of modern society is the striving for flexibility and mobility in all areas of the daily life. Worldwide a steady rising of traffic can be noticed. In the EU, since many years the traffic increases much stronger than the production capacity. This traffic growth is dominated by the road haulage [1].

Because of its efficiency and robustness the diesel engine became the dominating propulsion principle for trucks. The discussion about diesel exhaust related health effects led to a worldwide tightening up of the exhaust gas regulations, especially for heavy duty vehicles. Besides the emissions of particulate matter the limits for nitrogen oxides were substantially reduced. One possibility to keep these limits are engine measures. However, the problem is the trade-off between particulate matter and nitrogen oxides. Therefore secondary measures have to be used to fulfill the regulations. The SCR technique (selective catalytic reduction) is one method that is well proven to reduce nitrogen oxides. In the result nitrogen oxides could be reduced in principle by up to 95 % and particulate matter by up to 30 %. Gaseous ammonia (NH_3), ammonia in aqueous solution or urea in aqueous solution can be used as reducing agents [2-4].

Due to the increasing price of fossil diesel fuel more and more vehicles run on biodiesel (In Europe: mainly rapeseed oil methyl ester, RME). But the use of RME has specific demands to the engine and the exhaust gas aftertreatment system. To our knowledge no data are available about the influence of RME on SCR systems in heavy duty engines meeting the emission standard EURO IV, which were recently introduced to the market.

It was the goal of the investigations to figure out the influence of the maximum allowed phosphorus content of 10 ppm (European specification DIN EN 14214) on the long-term stability of the SCR system, because phosphorus acts poisonous to catalysts [5, 6]. However, it was not possible to buy RME with 10 ppm phosphorus on the German market. All qualities had significantly lower phosphorus concentrations. Therefore tributylphosphate was added to achieve a phosphorus content of 10 ppm.

Materials and Methods

Fuels

Four different fuels were tested: one common fossil diesel fuel (DF), two rapeseed oil methyl esters with high contents of phosphorus (RME_{10}), and one rapeseed oil methyl ester with a low (regular) content of phosphorus (RME), which was solely tested in the ESC-Test. The second batch of RME_{10} (RME_{10b}) was tested after 800 operating hours of the catalyst in an endurance test. It was tested for further 200 operating hours. The high content of phosphorus was obtained by adding tributylphosphate.

Engine and Test Conditions

A modern IVECO diesel test-engine, type Tector (F4A) with turbocharger and charge-air cooling was used. Technical data of this engine are given in Table 1.

Table 1. Technical data of the engine IVECO F4A.

Piston Stroke	120 mm
Bore of cylinder	102 mm
Number of cylinders	6
Engine displacement	5900 cm³
Rated speed	2500 min^{-1}
Rated power	220 kW
Maximum torque	1050 Nm at 1400 min^{-1}
Compression ratio	17:1

Measurements have been carried out on a SCR test system, type $SINO_x$ (Argillon). The SCR test system consists of the catalyst, a controlling device and a dosing unit for the reducing agent. A 32.5% aqueous urea solution (DIN 70070), also known as AdBlue™, was used as reducing agent. The SCR catalyst is a fully extruded, fine-cellular honeycomb catalyst consisting mainly of titanium oxide, doped with vanadium as activity enhancing substance. Emissions were measured before and after the catalyst. Additionally measurements were carried out on a reference catalyst that was not used with RME_{10}.

The engine was operated at two different test modes to determine the durability of the SCR in the so-called E46 Endurance Test evolved by IVECO. The modes changed between high idling and rated power. Strong jumps of the exhaust gas temperature were reached and represented high stress for the SCR system. For the measurement of the regulated and non-regulated emissions the 13-mode ESC test was used.

Results

RME_{10} showed advantages for all regulated emissions in comparison to DF with the exception of NO_x. Even with the brand new SCR system (0 hours) the limit for NO_x (EURO IV: 3.5 g/kWh) is exceeded (Fig. 2), since the dosing calibration was kept unchanged for the operation with RME_{10}.

All fuels were within the EURO IV limit for CO of 1.5 g/kWh. RME_{10} showed almost 50 % lower emissions compared with DF (Fig. 1). Significant differences before (0 hours) and after the endurance test (1000 h) could not be determined for RME_{10}.

In contrast to the CO emissions the aging of the catalyst had a significant impact on NO_x (Fig. 2). Moreover, the high phosphorus content damaged the SCR system: After running 1000 hours on RME_{10}, even with DF the limit of 3.5 g/kWh was exceeded. Simultaneously the slip of ammonia increased from 13 ppm to 79 ppm for RME_{10} and from 23 ppm to 94 ppm for DF, data not shown.

RME_{10} emitted 55% less unburned hydrocarbons than DF (Fig. 3). However, all results were very well within the EURO IV limit of 0.46 g/kWh.

The catalyst lost activity during the endurance test regarding NO_x, HC, and PM (Figs. 2-4).

Diesel fuel led to a PM increase. The endurance test had a further negative effect on the activity regarding PM. For both fuels the emissions increased after 1000 hours on RME_{10}. Since engines are designed for DF, more unburned fuel is emitted when running on RME. This was verified by several studies [10-13].

It was not possible to measure the particle size distribution at the beginning of the project. So after the 1000 hours endurance test a brand new SCR system was used as reference. Additionally RME without phosphorous additive was used for comparison. Particle size distribution was measured before and behind the SCR system.

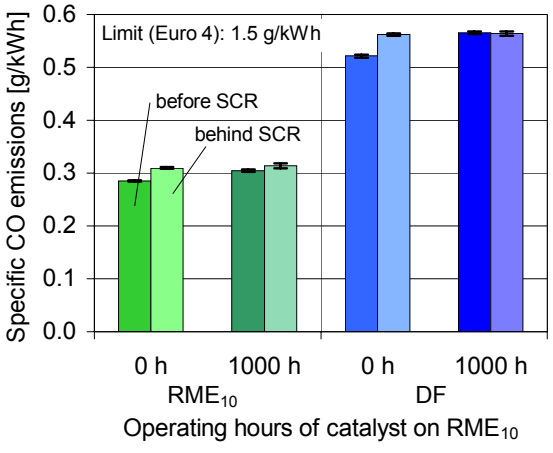

Figure 1. Specific CO emissions; ESC-Test.

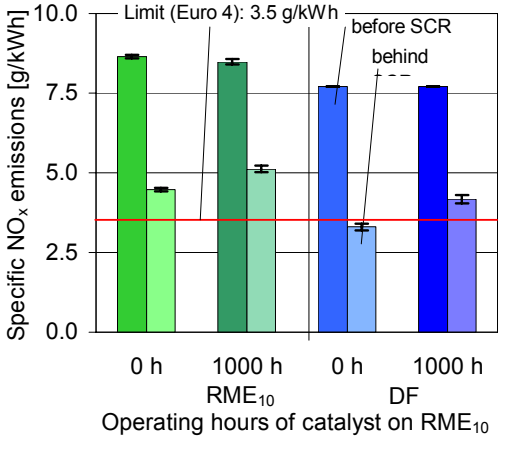

Figure 2. Specific NO_x emissions; ESC-Test.

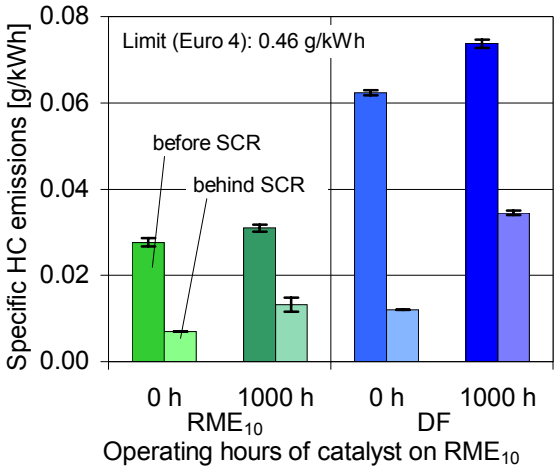

Figure 3. Specific HC emissions; ESC-Test.

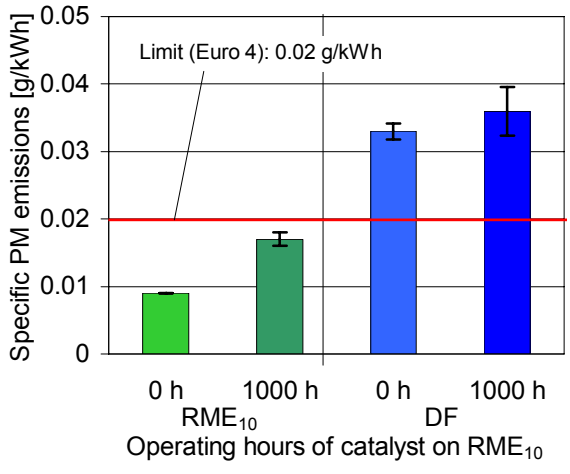

Figure 4. Specific PM emissions; ESC-Test.

The particle size distribution was measured by SMPS (Fig. 5). RME shows lower emissions than diesel fuel. It is striking that RME_{10} leads to severe increases of ultra fine particle emissions, as revealed by sampling before the SCR system (symbol "pre cat."). Furthermore, after 1000 hours of engine operation with high-phosphorous RME_{10}, performance of the SCR was substantially deteriorated. This is evident from comparing distribution curves "1000 h" and "reference". No such influence of the SCR on counts of finest particles was observed both for DF and regular RME. Thus, fuel quality seems to be mainly responsible for higher ultra fine particle emissions.

Generally, the mutagenic potency of all PM extracts was low and was further reduced in assays with metabolic activation (Fig. 6). Significant differences of mutagenicity between extracts of the used fuels were not observed. The numbers of mutations with and without metabolic activation were much lower in relation to earlier investigations without SCR catalyst indicating a probably very effective reduction of PAH and especially nitrated PAH of the system and a decreased health risk, respectively [14-20]. Nevertheless, the endurance test led to an increase of the number of mutations.

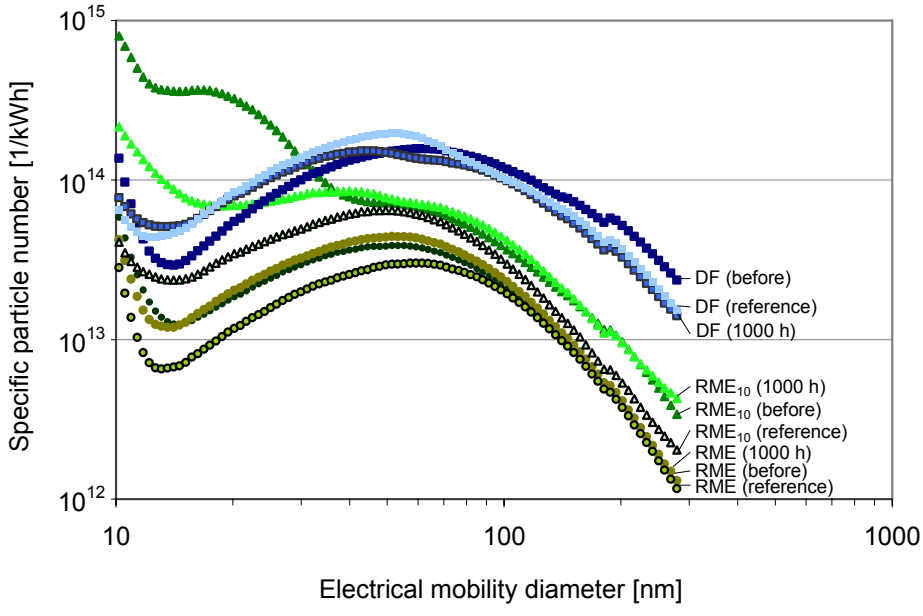

Figure 5. Size distribution of particles with respect to number of particles (SMPS).

Conclusion

Exhaust gas emissions from a modern diesel engine (class EURO IV) with SCR system were measured with common diesel fuel, two rapeseed oil methyl esters with high contents of phosphorus and one rapeseed oil methyl ester with a low content of phosphorus (as found on the market). It was the goal to investigate the influence of phosphorus on the function of an SCR system.

At the example of this test engine and the used SCR system, the NO_x limit was exceeded with biodiesel.

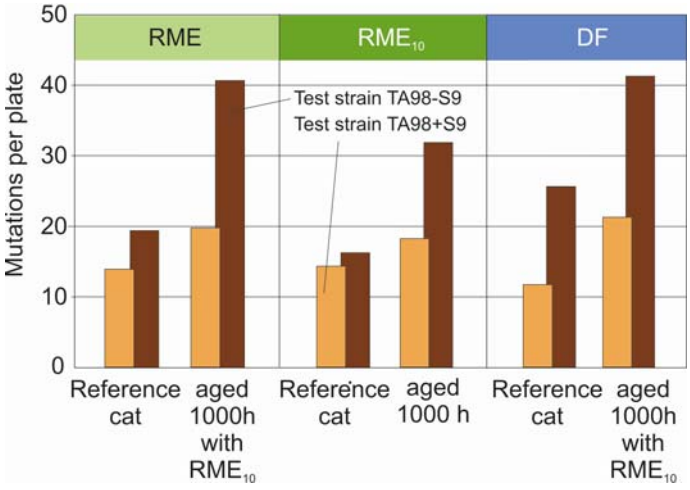

Figure 6. Mutagenic effects of PM extracts with (+S9) and without (-S9) metabolic activation. ESC tests on IVECO Tector F4A engine.

The content of phosphorus in biodiesel has a significant influence on the emissions of the regulated and non-regulated emissions. Phosphorus acts poisonous to the catalyst and leads to a decrease of activity. The ultra-fine particle emissions increased by the influence of phosphorus. The mutagenic potency was on a low level for both diesel fuel and biodiesel. However, the aging of the SCR system led to an increase of mutations.

In summary, the use of biodiesel with low phosphorus content is strongly recommended. The European maximum value of 10 ppm should be lowered. In the meantime the biodiesel producers should continue to produce fuel with phosphorus concentrations of a lower limit than required by the specification. OEMs should not allow 10 ppm phosphorus for their SCR systems.

Acknowledgements

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References

[1] Umweltbundesamt 2005. Der Beitrag der Lkw-Maut zu einer nachhaltigen Mobilität. Positionspapier des Umweltbundesamtes zur Novellierung der EU-Wegekostenrichtlinie. Berlin.
[2] Amon, B., Fischer, S., Hofmann, L., Zürbig, J. 2004. SCR – A technology for global emissions control of diesel engines. SAE-Paper F2004V160.
[3] Brodrick, C.J., Farsh-chi, M., Dwyer, H.A., Sperling, D., Gouse, S.W., Doelling, W., Hoelzer, J., Jackson, M. 1999. Urea-SCR system demonstration and evaluation for heavy-duty diesel trucks. SAE-Paper 1999-01-3722
[4] Hüthwohl, G., Li, Q., Lepperhoff, G. 1993. Untersuchung der NO_x-Reduzierung im Abgas von Dieselmotoren durch SCR-Katalysatoren. *MTZ* 54, 310-315.
[5] Remmele, E., Wilharm, T. 2000. Stand der Standardisierung und der Forschungsarbeit sowie neue Erkenntnisse aus der Analytik. Protokoll zur 6. Sitzung des LTV-Arbeitskreises „Dezentrale Pflanzenölgewinnung“, Straubing.
[6] StMLU 2002. Pflanzenölbetriebene Blockheizkraftwerke Leitfaden. Aus der Reihe Materialien Umwelt & Entwicklung Bayern, Augsburg.
[7] Ames, B.N., McCann, J., Yamasaki, E. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research* 31, 347-363.
[8] Maron, D.M., Ames, B.N. 1983. Revised methods for the Salmonella mutagenicity test. *Mutation Research* 113, 173 – 215.
[9] Matsushima, T., Sawamura, M., Hara K., Sugimura, T. 1976. A safe substitute for polychlorinated biphenyls as an inducer of metabolic activation system, in: Serres, F.J., Fouts, J.R., Bend, J.R., Philpot, R.M. (Eds.), In vitro metabolic activation in mutagenesis testing. Elsevier/North-Holland, Amsterdam, 85-88.
[10] Krahl, J., Bünger, J., Munack, A. 1998. Biodiesel exhaust emissions and determination of their environmental and health effects; in: Plant oils as fuels: present state of science and future developments. Springer, Berlin, 104 – 122.
[11] Bagley, S.T., Gratz, L.D., Johnson, J.H., McDonald, J.F. 1998. Effects of an oxidation catalytic converter and a biodiesel fuel on the chemical, mutagenic, and particle size characteristics of emissions from a diesel engine. *Environ. Sci. Technol.* 32: 1183-1191.
[12] Munack, A., Schröder, O., Stein, H., Krahl, J., Bünger, J. 2003. Systematische Untersuchungen der Emissionen aus der motorischen Verbrennung von RME, MK1 und DK. *Landbauforschung Völkenrode*, special issue 252.
[13] Bünger, J., Krahl, J., Franke, H.U., Munack, A., Hallier, E. 1998. Mutagenic and cytotoxic effects of exhaust particulate matter of biodiesel compared to fossil diesel fuel. *Mutation Research* 415, 13 – 23.
[14] Schwarz, S. 2005. Einfluss von Biodiesel auf ein SCR-Abgasnachbehandlungssystem. Diploma thesis, University of Applied Sciences, Coburg, Germany 2005.
[15] Bünger, J., Müller, M.M., Krahl, J., Baum, K., Weigel, A., Hallier, E., Schulz, T.G. 2000a. Mutagenicity of diesel engine particles from two fossil and two plant oil fuels. *Mutagenesis* 15, 391 – 397.
[16] Bünger, J., Krahl, J., Baum, K., Schröder, O., Müller, M., Westphal, G., Ruhnau, P., Schulz, T., Hallier, E. 2000b. Comparison of diesel engine emissions from biodiesel and petrol diesel fuel: Particle size and number analysis, cytotoxic and mutagenic effects. *Arch Toxicol* 74, 490 – 498.
[17] Bünger, J., Krahl, J., Weigel, A., Schröder, O., Brüning, T., Müller, M., Hallier, E., Westphal, G. 2006. Influence of fuel properties, nitrogen oxides, and exhaust treatment by an oxidation catalytic converter on the mutagenicity of diesel engine emissions. *Arch Toxicol* 80, in press.
[18] Krahl, J., Bünger, J., Munack, A., Bahadir, M., Schröder, O., Stein, H., Dutz, M. 2003a. Biodiesel and Swedish low sulfur diesel fuel as ecologically compatible fuels in modern diesel engines. *Fresen. Environ. Bull.* 12, 640-647.
[19] Krahl, J., Munack, A., Schröder, O., Stein, H., Bünger, J. 2003b. Influence of biodiesel and different designed diesel fuels on the exhaust gas emissions and health effects. Society of Automotive Engineers, SAE 2003 Transactions, Journal of Fuels and Lubricants 112, Warrendale, PA, USA, 2447-2455.
[20] Krahl, J., Munack, A., Schröder, O., Stein, H., Herbst, L., Kaufmann, A., Bünger, J.: Fuel design as constructional element with the example of biogenic and fossil fuels. Agricultural Engineering International. Vol. VII, Manuscript EE 04 008, http://cigr-ejournal.tamu.edu (2005)

Effects of fatty acids on oxidation stability and low temperature fluidity of biodiesel

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Abstract

In this study, the composition and content of the fatty acids methyl esters of biodiesel fuel samples, which were prepared from rapeseed oil, soybean oil, palm oil and waste frying oil, were analyzed. The oxidative stability of biodiesel was measured by Schaal Method and the low temperature fluidity of biodiesel was investigated. The technological parameters of biodiesel samples, such as peroxide value, acid value, TEP value, kinematic viscosity, cold filter plugging point and pour point, were also analyzed. The results showed that the higher content of the saturated fatty acidic methyl esters and the palm methyl ester in the fuel, the better the oxidative stability was; the higher the unsaturated methyl esters contents, the better the low temperature fluidity was; the high monounsaturated methyl esters contents improved both oxidative stability and low temperature fluidity of biodiesel.

Key words:biodiesel, fatty acids, oxidative stability, low temperature fluidity

Introduction

Biodiesel is defined to be the alkyl monoesters of vegetable oils, animal fats or recycled greases and used vegetable oils. Vegetable oils, such as soybean oil, rapeseed oil and tropical oils (palm oil and coconut oil) are the major sources of biodiesel. With these feedstock and alcohol (ethanol or methanol), transesterification reactions are carried out to produce monoesters known as biodiesel and glycerin in the presence of a catalyst. Because of its low cost, methanol is the most common alcohol used but ethanol is used in some cases. It is a renewable, biodegradable, cleaner burning alternative to petroleum fuels. Also biodiesel can be blended with diesel to form into a biodiesel blend which can be used in compression-ignition engines with little or no modifications (M.A. Kalam et al., 2002, Xu Ge et al., 2003).

Biodiesel as renewable biofuels has rapidly grown in the world market, but there are some problems which block its development. In storage time, the biodiesel fuel containing the polyunsaturated fatty acids such as linoleic acid and linolenic acid are inevitable to oxidation. The chemical changes in the fuel associated with auto-oxidation usually produce hydroperoxides, and then aldehydes and ketones, and the hydroperoxides can be also polymerized, which greatly reduce the performance of biodiesel (Sendzikiene E et al., 2005; Xu Ge et al., 2004, Robert O. Dunn, 2005). In addition to auto-oxidation of biodiesel, another drawback with the use of biodiesel is its poor cold flow properties compared with diesel fuel. In cold weather, some biodiesel will easily appear cloudy due to the wax crystals that can block the fuel lines and filters, because its cold filter plugging point, pour point and solid point are higher than diesel fuel (Sumit Tayal, 2006; Wu Miaoxin et al., 2005; Han Enshan et al., 2006). So vehicles running on biodiesel may experience more fuel system plugging problems than petroleum diesel fuel. It is necessary to enhance the storage stability and cold flow properties of biodiesel.

The oxidative stability and cold flow properties of biodiesel were studied in this paper. We used Schaal Method to investigate the oxidative stability of rapeseed methyl ester (RME), soybean methyl ester (SME), palm methyl ester (PME) and waste frying methyl ester (FWME), and determined their kinematic viscosity, cold filter plugging point and pour point; and we analyzed the effect of the profile of fatty acids methyl esters on The oxidative stability and cold flow properties of biodiesel.

Material and Methods

Materials

Crude rapeseed oil and soybean oil were purchased from Wuhan Zhongpai grain and oil Co., Ltd. Crude Palm oil was from the Wuhan food oil market, and waste frying oil was collected from some restaurants. Methanol analytical grade and sodium hydroxide analytical grade were supplied by Tianjin nankai Chemical Co., Ltd. . The Heptadecanoic acid methyl ester was obtained from Sigma－Aldrich Co..

Methods

Experimental methods

A sample of 1000 g crude oil was transferred to a two-neck flask equipped with a thermometer and a reflux condensator; and the oil was heated and stirred by a bar until the desired temperature was reached. A mixture of methanol and sodium hydroxide were added to the oil and the transesterification reaction began at 75°C. The reactor was kept at 75°C for 2 h. The procedure was carried out using a molar ratio of methanol/ crude oil which were 5 and a catalyst quantity equivalent to 1.0 %

mass of oil. At the end of the reaction period, the glycerol rich-phase was separated from the methyl ester layer in a decantation funnel. Then the latter phase was washed with a H_3PO_4 solution, and the biodiesel was separated from the H_3PO_4 solution. In the rotary evaporator, the remnant menthol in the biodiesel was distilled. The biodiesel was washed with hot water again, and the washed methyl esters were then dried with anhydrous sodium sulphate. The fatty acid composition of the samples was analyzed by GC.

The sample of 200 g of RME, SME, PME, FWME were transferred to the flasks which were put in the oven at 63°C±1°C., and the peroxide value, acid value, TEP value, kinematic viscosity, cold filter plugging point and pour point of biodiesel were determined in a time-interval

Analyses

The peroxide value(POV), acid value(AV), kinematic viscosity(KV) were examined at 40°C. Cold filter plugging point(CFPP) and pour point(PP) were carried out according to GB/T 5538, GB/T 5530, GB/265, SH/T0248, GB3535-83,respectively. The thiobarbituric acid(TBA) value was determined as follows: a sample of 10 g oil was transferred to evaporator flask with a reflux condensator, and a mixture of an HCl solution and wet olefin were added to the oil; the sample in the flask was heated through the vapor for 10 min, and collected the fraction at one time. The water was added to the fraction in 50ml container, and a mixture of 5ml of 50ml fraction and 5ml TEP was heated for 30 min at 100°C. The mixture was cooled in 10min, and its absorbency was determined in 532nm.

The methyl ester composition was obtained by gas chromatography using a HP6890N as Chromatograph, with DB-17HT capillary column. The temperature program was as follows: 160°C for 0.5 min, heating until 173°C at a rate of 40°C/min and holding at 175°C for 1 min; then heating until 245°C at a rate of 7°C/min and holding for 5min. The injector was set up for 260°C, as well as the FID detector. Helium was used as carrier gas, at a flux of 45 ml/min. Methyl heptadecanoate was used as an internal standard.

Results

Fatty acid distribution of biodiesel

The fatty acid composition of these biodiesel was analyzed by HP6890N Gas Chromatograph. Table 1 shows that FWME and PME contains saturated fatty acid methyl ester which mainly is palm methyl ester, higher than those of SME and RME. SME and RME containing the mass of unsaturated fatty acid methyl ester that reach 84.4% and 93.3%, respectively; the mass of linoleic methyl ester of SME is 50.1% and the mass of monoenoic acid methyl ester of RME is 71.7%. It was also observed that the order of the content of 18-carbon fatty acid methyl ester of biodiesel was as follows: SME>PME>RME>FWME, and fatty acid methyl ester(carbon number >18) of SME reaches 40.3%.

Table 1 Relative content of fatty acid methyl ester in biodiesel

	FWME	SME	RME	PME
C12:0 (%)	0.2	/	/	0.2
C14:0 (%)	1.0	0.1	0.1	0.9
C16:0 (%)	43.7	10.8	3.2	39.4
C16:1 (%)	0.5	0.2	0.3	0.4
C17:0 (%)	0.1	0.1	0.1	0.1
C17:1 (%)	/	0.1	/	/
C18:0 (%)	3.8	3.9	1.3	3.7
C18:1 (%)	39.9	24.7	33.4	43.1
C18:2 (%)	8.7	50.1	14.5	11.3
C18:3 (%)	0.4	7.4	7.0	0.3
C20:0 (%)	0.4	0.4	0.6	0.3
C20:1 (%)	0.2	0.7	8.7	0.4
C20:2 (%)	/	/	0.3	0.2
C22:0 (%)	0.1	0.3	/	/
C22:1 (%)	0.1	1.2	29.3	/
C22:2 (%)	/	/	0.4	/
C24:0 (%)	/	0.2	1.0	/

Comparison of the oxidation stability of biodiesel

The oxidative stability of RME, SME, PME and FWME was investigated by Schaal method. As shown in Fig. 1, the POV of FWME reaches 5.47meq/kg in 14d, which changes smaller than those of other biodiesel, and the POV of RME, SME and PME improve 102.09 meq/kg, 30.17meq/kg and 79.86meq/kg, respectively. From Fig. 1, it may be concluded that the oxidative stability of FWME is greater than that of RME according to the POV index, because their POV in 1d are pretty much the same thing. Fig. 2 indicates that the AV of RME, SME, PME and FWME increase 1.99mg KOH/kg, 1.88mg KOH/kg, 1.07mg KOH/kg and 0.25mg KOH/kg respectively, but the first value of FWME is lowest; so it can imply that the oxidative stability of FWME is the best among the four biodiesel and the oxidative stability of SME is poor. As can be seen from Fig. 3, the change of the TAB value of the samples shows the similar result compared with Fig. 2; the TAB value of

FWME is stable and the TAB value of SME enhances 14.54 mg/kg.

Comparison of the cold flow properties and kinematic viscosity of biodiesel

CFPP and PP have been used to characterize the cold flow operability of biodiesel fuels. The four samples were placed in the oven at 63°C±1°C, and the value of CFPP, PP and KV were termly evaluated. Table 2 and Table 3 indicate that RME has the lowest CFPP and PP in these biodiesel, but the values obviously altered when RME was gradually oxidated. The CFPP and PP of RME enhance 4°C and 10°C in 40d. Also the CFPP of SME increased from -2°C to 2°C and PP of SME increase from -3°C to -1°C, respectively. However, as shown in Table 2 and Table 3, the change of CFPP and PP of FWME and PME was inapparent as compared with RME and SME. The KV results at 40°C (Fig. 4) show that there is different variation among the four samples. The value of KV of SME increases from 4.629mm^2/s to 9.169mm^2/s, but that of PME, RME and FWME enhance 0.949mm^2/s,0.676mm^2/s and 0.527mm^2/s. It can be found that the results of the changes of KV, AV, TBA and CFPP are similar.

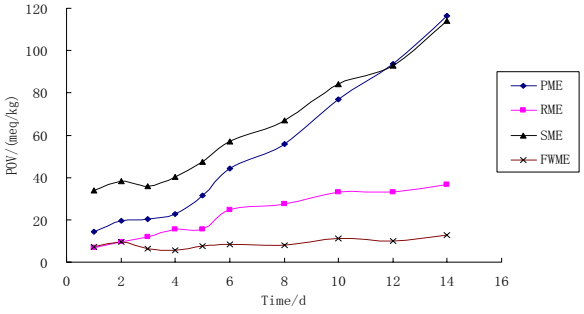

Fig. 1 Comparison for POV of four biodiesel

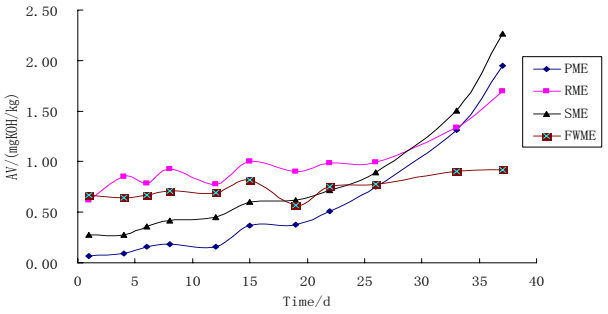

Fig. 2 Comparison for AV of four biodiesel

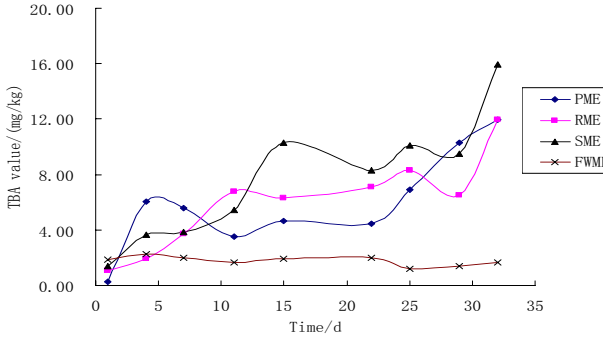

Fig. 3 Comparison for TBA value of four biodiesel

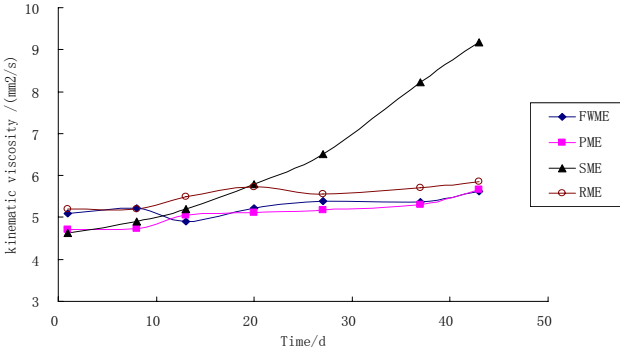

Fig. 4 Comparison for KV of four biodiesel

Table 2 Comparison for cold filter plugging point of four biodiesel (°C)

	FWME	PME	SME	RME
1d	11	11	-2	-11
20d	11	11	-1	-9
40d	12	11	2	-7

Table 3 Comparison for pour point of four biodiesel (°C)

	FWME	PME	SME	RME
1d	9	8	-3	-20
20d	9	9	-2	-17
40d	11	10	-1	-10

Discussion

One drawback of biodiesel is that biodiesel is prone to oxidation together with air exposure. Previous studies demonstrated that biodiesel stored at 4 and 20°C could degraded less than 10% within 52 weeks while nearly 40% degradation was found for those samples stored at a higher temperature, i.e. 40°C (D.Y.C. Leung et al.2006). Saturated compounds (C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid) have higher cetane numbers and are less prone to oxidation than unsaturated compounds but they tend to crystallize at unacceptably high temperatures (Mustafa Canakci, 2007).

As shown in the results, Biodiesel from soybean oil is highly unsaturated so its cold low properties are acceptable, for they have more linoleic methyl ester. However, it is more prone to oxidation than PME and WFME containing high saturated which has more palmtic methyl ester. RME has the best cold low properties among these biodiesel and good anti-oxidation performance relatively. It has been reported that the good order of biodiesel was RME>SM E>CME (cotton methyl esters) for the low temperature fluidity by compositions, solidifying point and CFPP (Xu Ge, 2004).The low-temperature fluidity of the biodiesel fuel depended on the content and distribution of the saturated fatty acid methyl esters in the fuel:the higher the esters content and the longer the carbon chains in the fuel, the worse the low-temperature fluidity was (Wu Miaoxin et, al.,2004).

Conclusions

The following conclusions may be drawn from the above study: the higher the saturated methyl esters and the palm methyl ester content in the fuel, the better the oxidative stability was; The higher the unsaturated methyl esters contents, the better the low temperature fluidity was; the high monounsaturated methyl esters contents improved the oxidative stability and low temperature fluidity of biodiesel.

References

[1] M.A. Kalam, H.H. Masjuki. (2002). Biodiesel from palmoil—an analysis of its properties and potential. Biomass and Bioenergy, 23,471-479.
[2] Xu Ge, Wu Guoying. (2003). The investigation of blending properties of biodiesel and No.0 diesel fuel. Journal of Jiangsu Polytechnic University, 15(2), 16-18.
[3] Sendzikiene E, Makareviciene V, Janulis P. (2005).Oxidation stability of biodiesel fuel produced from fatty wastes. Polish Journal of Envirmental Studies, 14(3), 335-339
[4] Xu Ge, Wu Guoying, Yu Juan. (2004). Investigation of biodiesel oxidative stability. New Chemical Materials, 32(2), 9-31
[5] Robert O. Dunn. (2005). Effect of antioxidants on the oxidative stability of methyl soyate (Biodiesel).Fuel Processing Technology, 86(10), 1071-1085
[6] Sumit Tayal. (2006). Detection of cold flow properties of diesel and biodiesel fuel using optical sensor, University of Missouri-Columbia.
[7] Wu Miaoxin, Wu Guoying, Xuan Hui, et, al.. (2005). Influencing factors of low-temperature flowing properties of soybean oil. Journal of Fuel Chemistry and Technology, 33(6), 688-702.
[8] Han Enshan, Kang Hongxin, Wei Zihai, et, al.. (2006). The Study on the low-temperature fluidity and the progress of pour point depressant of bio-diesel. Chemical Intermediate, 1, 13-17.
[9] D.Y.C. Leung, B.C.P. Koo, Y. Guo.(2006). Degradation of biodiesel under different storage conditions. Bioresource Technology, 97,250-256.
[10] Mustafa Canakci.(2007), The potential of restaurant waste lipids as biodiesel feedstocks. Bioresource Technology, 98,183-190.
[11] Xu Ge, Wu Miaoxin, Wu Guoying.(2004). Study of the low temperature flowing properties of biodiesel, Journal of Jiangsu Polytechnic University, 16(4),14-16.
[12] Xu Ge, Wu Guoying. (2004). Comparison in oxidative stability of biodiesel fuel from rapeseed oil and 0# diesel fuel. China Oil & Fat, 29(12),71-73.
[13] Wu Miaoxin, Wu Guoying, Han Lifeng, et, al.. (2004). Low-temperature fluidity of bio-diesel fuel prepared from edible vegetable oil, Petroleum Processing and Petrochemicals, 36(4), 57-60.

Processing of oilseeds in decentralised oil mills in Germany —results of a survey

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Abstract

Processing of oilseeds in decentralised oil mills becomes increasingly more important in Germany. Since 1999 the number of decentralised oil mills in Germany nearly has been tripled. In addition to the 79 existing plants in 1999 [1], the number of decentralised oil mills has grown since than by 140 (state of March 2004). In total about 219 plants are admitted until 2004 in the federal territory (figure 1). Against this background the Association for Technology and Structures in Agriculture initiated a survey in cooperation with the Technology and Support Centre of Renewable Raw Materials (TFZ) with the title "processing of oilseed in decentralised oil mills". Goal of the investigation was to do a stock-taking of the present status quo of oil mills' technical equipment. Furthermore, the investigation was supposed to seize mass flows of the assigned raw materials and the produced products and to determine the importance and relevance of the branch of business "processing of oilseed in decentralised oil mills".

Key words: cold pressed vegetable oil, rapeseed, rapeseed oil, decentralised oil mill

Introduction

On behalf of the Association for Technology and Structures in Agriculture (KTBL) and financially supported by the Union for the Promotion of Oil and Protein Plants (UFOP) the Technology and Support Centre of Renewable Raw Materials (TFZ) conducted in March 2004 a written survey about "processing oilseed in decentralised oil mills". The questionnaire contained 10 pages with nine main topics and has been send to 243 interviewees. The return rate amounted to 100 questionnaires (41 %). 90 questionnaires (37 %) met the criteria to be used in the survey, even though some of them were only partially completed. Information which has been asked besides general information regarding the operating company, were above all the individual equipment components, like seed preparation, pressing technique, oil cleaning technology and oil storage. Additionally data about quality of seed and oil, price information and proceeds, as well as information on sales and distribution was collected.

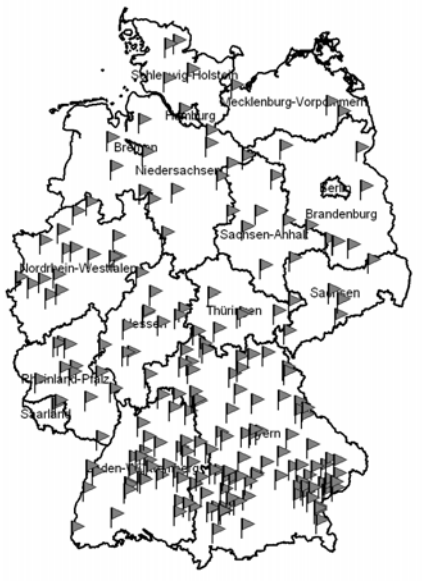

Figure 1: Locations of the 219 decentralised oil mills in Germany (state: March 2004)

Approximately 60 % of the decentralised oil mills (129 plants) are located in the states of Bavaria and Baden-Wurttemberg. An increase of decentralised oil mills can be noted above all for the states of North Rhine-Westphalia, Lower Saxony, Rhineland-Palatine and Brandenburg. New locations in the eastern states, especially in Thuringia, Saxony and Saxony-Anhalt are also to register. Overall a concentration of oil mills in the south of Germany, but also an increase in East

Germany is obvious. Only a few oil mills are resident in the states of Schleswig-Holstein and Mecklenburg-Western Pomerania.

Since 1990 most of the decentralised oil mills have been started-up. Only 2 % of the participating oil mills are older. Between 1990 and 1995 around 20 % of the plants were built. The date of commissioning of 29 % of the participating oil mills is between 1995 and 2000. A growing increase followed in the years 2001 to 2004, in this period 49 % of the participating oil mills were established (figure 2).

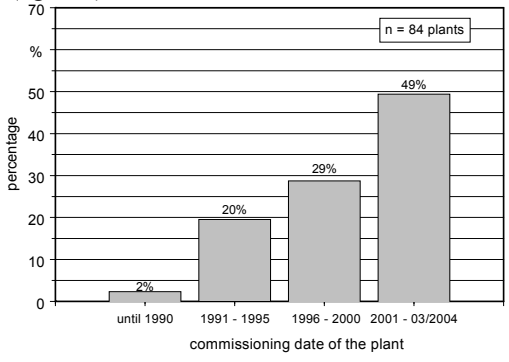

Figure 2: Commissioning date of the oil mills

Results

1. Processing Capacity and Production Emphasis: 60 decentralised oil mills processed in the year 2003 about 104.000 t of rapeseed. This amount of seed is equivalent to 35.000 t of rapeseed oil and to 69.000 t of press-cake. The extrapolation of the quantity up to 219 decentralised oil mills results in 380.000 t of rapeseed, what is equivalent to 128.000 t of rapeseed oil and 252.000 t of press-cake, respectively. This amount of seed is equivalent to 10 % of the rapeseed harvest in Germany in 2003.

41 % of the participating oil mills are processing up to 50 kg seed per hour. Around 43 % of the decentralised oil mills work with throughput rates from 50 kg to 150 kg per hour or with rates between 150 kg and 500 kg. Processing capacities over 500 kg and over 1000 kg per hour, respectively, are represented by 16 % of the plants (figure 3).

The production emphasis of nearly half of the participants is rapeseed oil fuel for adapted diesel engines, followed by the products edible oil and oil for feeding animals. Other applications like rapeseed oil as a raw material for the biodiesel production, motor oil, hydraulic oil or oil for chain saws were also named. Some oil mill operators process press-cake not only as a by-product, but as a main product.

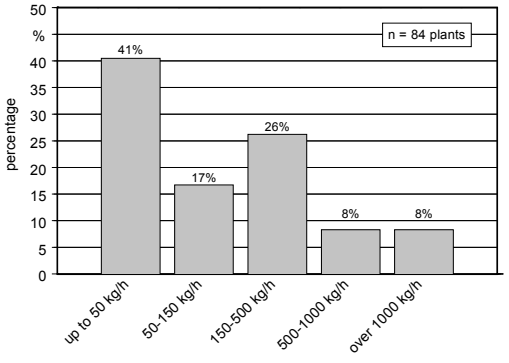

Figure 3: Processing capacity of the German decentralised oil mills

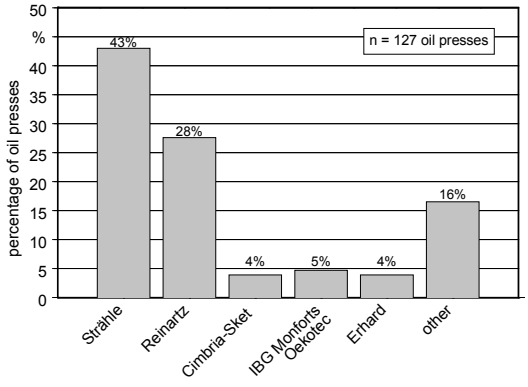

Figure 4: Manufacturer of oil presses used in decentralised oil mills in Germany

2. Technical Plant Units and Equipment: Around 59 % of the participating oil mills.

possess technical plant units for seed conditioning. Components for drying and purifying the seed before processing are represented in 70 % and 50 % of the decentralised oil mills, respectively. An additional foreign body separation is in 73 % of the plants in use. Only 18 % of the oil mill operators crush the oilseed before processing to oil.

Oil presses from the companies Karl Strähle GmbH & Co. KG and from the Maschinenfabrik Reinartz GmbH & Co. KG are mainly installed in the decentralised oil mills. Further manufacturer, like Cimbria-Sket, IBG Monforts Oektotec and Import Erhard are also represented. Other producers appear with smaller numbers of oil presses. Especially oil presses with throughput rates up to 50 kg seed per hour and over 50 kg up to 150 kg seed per hour are build-in. Figure 4 shows the distribution of the oil presses per manufacturer.

The purification of the muddy oil, as an important segment in the process flow, is conducted in different ways in the participating oil mills. One quarter of the mill operators work just with one-step purification, the main purification. Over 50 % of the operators purify oil in a second step with a safety filter. Another quarter the participants use not only one safety filter, but also or more safety filters to purify the oil. For the main purification mainly chamber filter-presses and vertical pressure leaf filters are in use. 38 % of the oil mills are working with a continuous sedimentation. 62 % of the participants have installed bag filters and cartridge filters for the safety filtration.

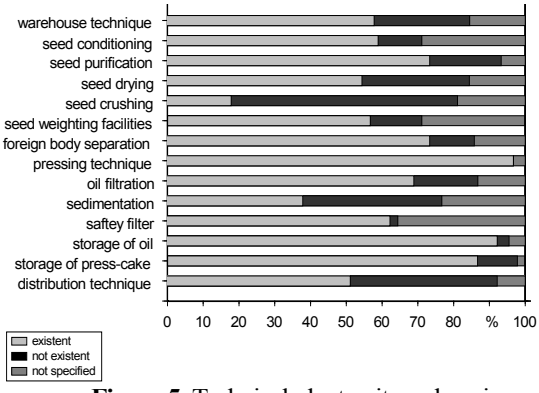

Figure 5: Technical plant units and equipment

In general 92 % of the oil mill operators store the output product rapeseed oil in different kind of tanks or containers. The favourite types for storing oil are IBC - containers made of poly-ethylene or tanks made out of steel. The by-product press-cake is usually stored locally in boxes or on stock for a short time.

Over 50 % of the decentralised oil mills possess as distribution technique a filling station or a tank truck for sale the oil. The processed oil and press-cake is collect by the customers as well as it is delivered by the oil mill operators.

A comprehensive overview of the technical plant units and equipment is given in figure 5.

3. Quality and Quality Assurance: Around 60 % of the participating oil mill operators are working in compliance with the "Quality Standard for Rapeseed Oil as a Fuel (RK-Qualitätsstandard 05/2000)". The RK-Quality Standard was worked out by the Technology and Support Centre for Renewable Raw Materials (TFZ) (http://www.tfz.bayern.de/) and describes a standard operation procedure for the production of rapeseed oil fuel. Characteristic and variable properties and their limiting values, for example the contamination, the acid value or the oxidation stability of the oil, are defined [3]. The RK-Quality Standard is 2006 replaced by the new pre-standard DIN 51605 [4].

83 % of the oil mills, which produce mainly rapeseed oil fuel for adapted diesel engines comply with the RK-Quality Standard.

Nearly 70 % of the participants accomplish one or up to six analyses of the oil per annum. More than 7 or more than 12 analyses per annum are rarely. Table 1 shows the distribution of the number of analyses. Besides the requirements contamination, acid value, phosphorus content, water content and oxidation stability other properties like content of sulphur, residues of pesticides, fatty acid composition or the content of vitamin E were analysed.

Table 1: Implementation of analyses of rapeseed oil (n = 64 plants)

Number of analyses	1 - 6 p.a.	7 - 12 p.a.	>12 p.a.
Percentage	69 %	15,5 %	15,5 %

4. Distribution and Sale: The provenance of the processed oilseed is mainly regional. About 13 % of the participants are using their own seed. Approx. 55 % of the oil mill operators are buying the seeds in regions not farer away than 25 km. Seed from a distance between 25 and 50 km is bought by 20 % of the participants.

58 % of the processed oil is distributed as raw material for the biodiesel production. Rapeseed oil fuel for adapted diesel engines (22 %), oil for feeding animals (14 %) and edible oil (1 %) are other utilisation forms. Around 11 % of the participating oil mill operators use their oil by themselves, for example in their tractors or in combined heat and power stations (CHP). One quarter of the participants deliver the oil in a radius up to 25 km and an other quarter even up to 50 km. Delivery in regions farer away than 100 km is offered by 36 % of the oil mill operators.

Around 11 % of the oil mill operators feed the obtained press-cake to their own cows, bulls or pigs. More than half of the participants deliver the press-cake into an area up to 25 km and 16 % up to 50 km. Delivery to places farer away is offered by 16 % of the oil mills. Besides the utilisation as a high-quality fodder for the agriculture, press-cake is also been sold to biogas plant operators.

5. Costs and Proceeds: Costs and proceeds for the input and output products vary extremely. Rapeseed edible oil, for example, is offered between 0.55 € and 5.00 € (zero rated for VAT) per litre. This wide range is traced back to the input of

conventional and ecological seed. The purchase quantity and the setting of distribution take influence of the pricing. Costs and proceeds of all input and output products are presented in table 2.

Table 2 Costs and proceeds of the input and output products of German decentralised oil mills (n = 55 plants), without VAT

Input/Output product	Unit	Mean	Median	Min.	Max.
Costs					
Rapeseed	€/ton	251	246	210	440
Proceeds					
Rapeseed oil as a fuel	€/ton	617	610	490	750
Fodder oil	€/ton	646	620	500	1,200
Edible oil	€/litres	2.1	1.54	0.55	4.98
Press-cake	€/ton	168	165	100	320

Summery

Since 1999 the number of decentralised oil mills in Germany nearly tripled. Around 50 % of the known 219 plants have been started-up between 2000 and 2004. The processing capacity of 40 % of the oil mills is up to 50 kg seed per hour. Throughput rates between 50 kg and 150 kg and also between 150 kg and 500 kg seed per hour are represented by further 40 % of the participating oil mills. Around 16 % of the decentralised oil mills processed more than 500 kg seed per hour. In 2003 an approximated amount of 380.000 t of rapeseed, based on a machine running time of 24 hours, on 250 days per year, was processed in 219 decentralised oil mills. This amount is equivalent to 10 % of the German rapeseed harvest in 2003. The provenance of the oil seed was mainly regional, only a small amount of the seed was bought from distances further than 100 km. The output products oil and press-cake was sold mainly locally. Half of the oil mill operators declared rapeseed oil fuel for adapted diesel engines as their main production emphasis followed by edible oil and oil for feeding animals. Other utilisations for rapeseed oil are technical oils like motor oil, hydraulic oil or rapeseed oil as a raw material for the biodiesel production.

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References

[1] Brenndörfer, M. (1999): Dezentrale Ölsaatenverarbeitung – KTBL-Arbeitspapier 267. Münster-Hiltrup: Landwirtschaftsverlag GmbH (130 pages).

[2] Stotz K. and E. Remmele (2005): Daten und Fakten zur dezentralen Ölgewinnung in Deutschland. Ed.: Technology and Support Centre of Renewable Raw Materials (TFZ), Straubing, Germany. Berichte aus dem TFZ 3 (53 pages).

[3] Remmele, E. (2002): Standardisierung von Rapsöl als Kraftstoff – Untersuchungen zu Kenngrößen, Prüfverfahren und Grenzwerten. Dissertation: Technische Universität München. Arbeitskreis Forschung und Lehre der Max-Eyth-Gesellschaft Agrartechnik im VDI Nr. 400 (194 pages).

[4] Deutsches Institut für Normung e.V. (2006): DIN V 51605: Fuels for vegetable oil compatible combustion engines - Fuel from rapeseed oil - Requirements and test methods. Beuth-Verlag GmbH.

The advancement on the preparation of biodiesel using rapeseed oil as material

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Abstract

Compared with other plant origin oils like vegetable oil or tree borne oil seeds, rapeseed oil has an ideal and optimum composition of fatty acids regarding to engine technology for the production of high-quality biodiesel. Transesterification is the most important procedure for the preparation of biodiesel. The catalyst of transesterification from rapeseed oil to biodiesel was reviewed in this paper including the liquid acid and base catalysts, solid acid and base catalysts, lipase catalyst and nano catalyst. Catalyzing the transesterification by nano solid base will be a promising research area for the production of biodiesel.

Key words: rapeseed oil, biodiesel, transesterifiction, catalyst

Introduction

In recent years, the world has been confronted with energy crisis due to the depletion of resources and the increasing environmental problems. The situation has led to the search for an alternative fuel, which should be not only sustainable but also environment friendly. One possible alternative to fossil fuel is the use of plant origin oils like vegetable oils and tree borne oil seeds (Zhao et al., 2006). Rapeseed oil has an ideal and optimum composition of fatty acids regarding to engine technology for the production of high-quality biodiesel. In the process for the preparation of biodiesel using rapeseed oil as material, the key technology is the transesterification of triglycerides to fatty acid alkyl esters and glycerol. The catalyst of transesterification from rapeseed oil to biodiesel was reviewed in this paper including the liquid acid and base catalysts, solid acid and base catalysts, lipase catalyst and nano catalyst. (Karmee & Chadha, 2005; Shah et al., 2006; Zhu et al., 2006).

Rapeseed oil-the important material for product biodiesel

In 1898, Rudolph Diesel first demonstrated his compression ignition engine at the World's Exhibition in Paris, in which peanut oil was used as the original biodiesel. However, it is difficult for the vegetable oil (e.g. pure rapeseed oil) to be applied directly as a cheap and convenient alternative in conventional Diesel, because some technical problems existed such as high density, viscosity, poor filtration and low volatility. So it needs some sort of modification before vegetable oil is used as substitute of conventional fuels.

Rapeseed oil is a very important source not only of the edible-oil technology but also of biodiesel technology. It has an ideal and optimum composition of fatty acids with regard to engine technology for the production of high-quality biodiesel. And the by-product, rape meal and rapeseed cake, is a high quality protein feed ingredient which can be used instead of soy meal, particularly in cattle rations. Therefore rapeseed oil is a superior oil stock for biodiesel production in most of European countries, partly because rapeseed produces more oil per unit of land area compared to other oil sources, such as soy beans (Xie et al., 2007). However, rapeseed biodiesel costs more than standard diesel fuel in the costs of growing, crushing and refining rapeseed biodiesel. Prices of rapeseed oil are of fairly high levels at present because of the increasing demand on rapeseed oil for this purpose, although world production is growing rapidly, with FAO reporting that 36 million tonnes of rapeseed was produced in the 2003-2004 season, and 46 million tonnes in 2004-2005 season.

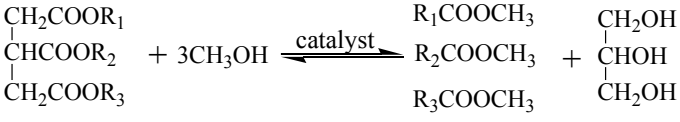
Some high-performance rapeseed varieties have been developed to resolve the resource problem in China. The new rapeseed variety was called double-zero type, which is low in glucosinolates (bitter principals) and practically free of erucic acid. In China, double-zero rapeseed was produced in large volumes since the mid 1990s. Rapeseed production is primarily concentrated in the Yangtzi valley region. In particular, some universities in China, such as Huazhong Agricultural University, have developed high-performance rapeseed varieties. These research groups have established a novel technique of molecule labeling method to assisting the selection of oilseed rape, which is applied in the utilization of oilseed rape breeding, and built up several breeding varieties of Hybrid Brassica napus L oilseed rape(Quijada et al., 2006; Qian, W. et al., 2006). This will largely enhance the efficiency of breeding and the yield of oilseed rape. Rapeseed "oil cake" is also used as fertilizer in China, which may be used for ornamentals, such as Bonsai (Ozcimen & Karaosmanoglu, 2004). Thus it is promising for the preparation of biodiesel using rapeseed oil as material in China.

Biodiesel Preparation

The rapeseed oils contain free fatty acids, phospholipids, sterols, water, odourants and other impurities, which cannot be used as fuel directly. To solve these problems, the rapeseed oil requires slightly chemical modification, such as transesterification, pyrolysis and emulsification. Transesterification is the most important procedure for the preparation of

biodiesel using rapeseed oil as material. The reaction of transesterification and corresponding catalysts are the focus in below.

Rapeseed oil comprised of 98% triglycerides and small amounts of mono-and diglycerides (Arzamendi et al., 2006). Triglycerides are esters of three molecules of fatty acids and one of glycerol containing substantial amounts of oxygen in their structure. The fatty acids vary in their carbon chain length and in the number of double bonds (Jeong et al., 2004). In the process of transesterification, the first step is the conversion of triglycerides to diglycerides, followed by the conversion of diglycerides to monoglycerides, and of monoglycerides to glycerol, yielding one methyl ester molecule per mole of glyceride at each step (Dossin et al., 2006). The overall chemical reaction process is as follows:



Scheme.1 Transesterification of triglycerides

Up to now, the catalysts in transesterification of triglycerides include the liquid acid and base catalysts, solid acid and base catalysts, lipase catalysts and nano solid base catalysts as the novel one recently, which are described as follows.

1. The liquid acid and base catalyst

In the acid-catalyzed process, sulfonic acid and hydrochloric acid are often used as catalysts. However, the reaction time is very long (48-96h) even at reflux of methanol, and a high molar ratio of methanol to rapeseed oil is needed (30-150:1 mol/%). Several comprehensive studies have been reported based on the base catalyzed transesterification (Lopez et al., 2005). Also known as methanolysis, this reaction is commonly carried out in the presence of homogeneous base or acid catalysts. Potassium hydroxide, sodium hydroxide, and their carbonates, as well as potassium and sodium alkoxides such as $NaOCH_3$, are usually used as base catalysts for this reaction. The base catalysts are better than acid catalysts, due to the higher catalytic activity and less corrosive, which are most often used commercially. However, in the conventional homogeneous manner, removal of the base catalysts after reaction is a major problem because it wills product large amount waste water.

2. Solid acid catalyst

Strong liquid acid catalysts react slower and need higher reaction temperatures. Nonetheless, acid-catalyzed processes could produce biodiesel from low-cost feedstocks, lowering production costs. Thus solid acid catalysts have been developed to replace liquid acids. The corrosion and environmental problems associated with them could be avoided and product purification protocols reduced, significantly simplifying biodiesel production and reducing cost using solid acid catalysts. Sulfated zirconia (SO_4/ZrO_2) and sulfated tin oxide (SO_4^{2-}/SnO_2) have been shown to have applicability for several acid-catalyzed reactions (Lotero et al., 2005). Jitputti, Kitiyanan et al. catalyzed transesterification of crude palm kernel oil and crude coconut oil by different solid catalysts. In the case of SO_4^{2-}/ZrO_2, only 1 wt.% of this acidic solid was needed to catalyze the reaction, resulting in fatty acid methyl esters content higher than 90% (Jitputti, Kitiyanan et al., 2006). However, research dealing with the use of solid acid catalysts for biodiesel synthesis has been limited due to pessimistic expectations about reaction rates and unfavorable side reactions.

3. Solid base catalyst

Some solid base catalysts have been reported include hydrotalcite, alkaline-earth metals, carbamidine. Liu Xuejun et al. catalyzed the transesterification of soybean oil to biodiesel using SrO as a solid base (Liu, He et al., in press). And the same reaction catalyzed by potassium oxide loaded on alumina (Xie et al., 2006).There are many advantages to product biodiesel using solid base catalyst, such as vulnerable separated, easily realized automatic production, successive and repeatedly employed. But the reaction proceeded at a relatively slow rate in most of the experiments using heterogeneous catalysts. The reaction mixture constitutes a three-phase system, oil/methanol/catalyst, in which the reaction is inhibited because of the presence of heterogeneous catalysts.

4. Lipase-catalyzed transesterification

Recent studies (Turkan & Kalay, 2006) showed that biodiesel could also be produced enzymatically by lipase-catalyzed transesterification. In conventional chemical process for biodiesel production, alkali or acid is usually adopted as the catalyst. However, there are several problems associated with chemical processes such as difficulty in glycerol recovery, excessive energy cost, and the need for removal of catalyst from the product and so on. Enzymatic methods can solve these problems which allow mild reaction conditions and no chemical waste is produced. However it hasn't been reported that the technology of industrialization of lipase-catalyzed transesterification, because its price is very expensive and the catalytic efficiency of mono-lipase is very low.

5. Nano solid base catalyst

Nano solid catalyst particles have many advantages compared with general solid catalysts. For example, Nano solid catalysts have very small diameter, and large superficial area. These properties maybe solve the problem in application of general solid base, such as small reaction surface and strong resistance of transmitter. Recently, most researches about catalysts in transesterification concentrate in solid base. However nano solid base catalyst is applied in other reactions. In a word, very few researches were reported about nano solid base catalyst in the transesterification of biodiesel preparation. VenkatReddy, C. R. et al. reported that Soybean Oil and Poultry Fat to Biodiesel Catalyzed by Nanocrystalline Calcium Oxides. With their most active catalyst, deactivation was observed after eight cycles with soybean oil and after three cycles with poultry fat

(VenkatReddy, Oshel et al., 2006).

A novel nano-solid-base catalyst was prepared by our Lab, recently, which has been successfully applied to prepare biodiesel from rapeseed oil. The effects of varied factors on catalyst preparation have been analyzed by uniform design. More environmental friendly ant high efficiency catalysts will be researched in further work.

Conclusion

Because of producing double-zero rapeseed in large volumes, there will be enough materials for preparing biodiesel. Rapeseed biodiesel has become more attractive to replace petroleum fuel. Nowadays the traditional acid and base catalyst are still playing an important role in the technology of industrialization. The base catalysis is preferred to acid catalyst routes, which is often used commercially. Conventional homogeneous catalysts are expected to be replaced in the near future by environmentally friendly heterogeneous catalysts. Lipase-catalyzed and Supercritical transesterification have proceeded to the experiment of industrialization. Nano materials have present many particular properties in many fields. There will be a new approach to resolve the problem in catalyst. Green and environmental catalysts will be the important research field of catalytic chemistry. The nano solid base catalyzed transesterification is the promising area of research for production of biodiesel.

References

Arzamendi, G., E. Arguinarena, et al. (2006). Monitoring of biodiesel production: Simultaneous analysis of the transesterification products using size-exclusion chromatography. Chemical Engineering Journal 122: 31-40.

Dossin, T. F., M.-F. Reyniers, et al. (2006). Simulation of heterogeneously MgO-catalyzed transesterification for fine-chemical and biodiesel industrial production. Applied Catalysis B: Environmental 67: 136-148.

Hama, S., S. Tamalampudi, et al. (2006). Lipase localization in Rhizopus oryzae cells immobilized within biomass support particles for use as whole-cell biocatalysts in biodiesel-fuel production. Journal of Bioscience and Bioengineering 101: 328-333.

Jeong, G. T., D. H. Park, et al. (2004). Production of biodiesel fuel by transesterification of rapeseed oil. Appl Biochem Biotechnol 113-116: 747-58.

Jitputti, J., B. Kitiyanan, et al. (2006). Transesterification of crude palm kernel oil and crude coconut oil by different solid catalysts. Chemical Engineering Journal 116: 61-66.

Karmee, S. K. & A. Chadha (2005). Preparation of biodiesel from crude oil of Pongamia pinnata. Bioresource Technology 96: 1425-1429.

Liu, X., H. He, et al. Transesterification of soybean oil to biodiesel using SrO as a solid base catalyst. Catalysis Communications In Press, Accepted Manuscript.

Lopez, D. E., J. J. G. Goodwin, et al. (2005). Transesterification of triacetin with methanol on solid acid and base catalysts. Applied Catalysis A: General 295: 97-105.

Lotero, E., Y. Liu, et al. (2005). Synthesis of Biodiesel via Acid Catalysis. 44: 5353-5363.

Ozcimen, D. & F. Karaosmanoglu (2004). Production and characterization of bio-oil and biochar from rapeseed cake. Renewable Energy 29: 779-787.

Qian, W., J. Meng, et al. (2006). Introgression of genomic components from Chinese Brassica rapa contributes to widening the genetic diversity in rapeseed (B. napus L.), with emphasis on the evolution of Chinese rapeseed. Theor Appl Genet 113: 49-54.

Quijada, P. A., J. A. Udall, et al. (2006). Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (Brassica napus L.): 1. Identification of genomic regions from winter germplasm. Theor Appl Genet 113: 549-61.

Shah, S., A. Sharma, et al. (2006). Preparation of cross-linked enzyme aggregates by using bovine serum albumin as a proteic feeder. Analytical Biochemistry 351: 207-213.

Turkan, A. & S. Kalay (2006). Monitoring lipase-catalyzed methanolysis of sunflower oil by reversed-phase high-performance liquid chromatography: Elucidation of the mechanisms of lipases. Journal of Chromatography A 1127: 34-44.

Udall, J. A., P. A. Quijada, et al. (2006). Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (Brassica napus L.): 2. Identification of alleles from unadapted germplasm. Theor Appl Genet 113: 597-609.

VenkatReddy, C. R., R. Oshel, et al. (2006). Room-Temperature Conversion of Soybean Oil and Poultry Fat to Biodiesel Catalyzed by Nanocrystalline Calcium Oxides. 20: 1310-1314.

Xie, W., X. Huang, et al. (2007). Soybean oil methyl esters preparation using NaX zeolites loaded with KOH as a heterogeneous catalyst. Bioresource Technology 98: 936-939.

Xie, W., H. Peng, et al. (2006). Transesterification of soybean oil catalyzed by potassium loaded on alumina as a solid-base catalyst. Applied Catalysis A: General 300: 67-74.

Zhao, J., H. C. Becker, et al. (2006). Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theor Appl Genet 113: 33-8.

Zhu, H., Z. Wu, et al. (2006). Preparation of Biodiesel Catalyzed by Solid Super Base of Calcium Oxide and Its Refining Process. Chinese Journal of Catalysis 27: 391-396.

State and prospects of the production and use of rapeseed oil fuel in Germany

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Abstract

There is a growing demand on pure rapeseed oil, used as a fuel for adapted automotive and stationary diesel engines in Germany. The main reasons are increasing prices for fossil diesel fuel and heating oil as well as advantageous regulations for biofuels. Today 13 industrial and more than 300 decentralised oil mills produce rapeseed oil, mainly for energetic use in Germany. Oil mill capacities are being extended, a significant scale has been reached already. The demands on quality of rapeseed oil, used as a fuel in compatible diesel engines are defined in the new pre-standard DIN 51605. Quality assurance systems for decentral processed rapeseed oil are being developed and established. In Germany about 50 mainly small sized enterprises offer solutions for the technical adaptation of conventional diesel engines for the use of pure rapeseed oil. Experiences in field tests with rapeseed oil fuelled engines are mostly better than expected. The prior applications for rapeseed oil fuel are niche markets, such as environmental sensitive areas and agricultural machines, due to a limited number of engine types with high numbers of items and the advantage of closed economic and material flow loops.

Key words: cold pressed vegetable oils, rapeseed oil fuel, market implementation

Introduction

There is a growing demand on pure rapeseed oil, used as a fuel for adapted automotive and stationary diesel engines in Germany. Reasons therefore are increasing prices for fossil diesel fuel and heating oil, advantageous regulations for biofuels as well as the public awareness of the excellence of rapeseed oil fuel, regarding environmental impacts and the development of regional markets. However, pure rapeseed oil used as a fuel raises important questions about deficits and chances of extended utilisation.

It is the purpose of this work, to show the state of the art of pure rapeseed oil fuel production and utilisation under German conditions. Based upon these facts prospects for further expansion of rapeseed oil fuel applications in Germany will be deduced.

Comprehensive results of various research works are combined, to show the state of art and need for action. Important data are collected, to show the market relevance of rapeseed oil fuel in Germany and expert discussions are held, to asses the prospects of rapeseed oil fuel.

Results

1. General Framework: Rapeseed oil fuel, which is also known as Pure Plant Oil (PPO) is extracted and purified or refined oil of rapeseed (*Brassica napus*). The difference between rapeseed oil fuel and fatty acid methyl ester (FAME or biodiesel) is, that there is no step of transesterification for rapeseed oil production, which particularly enables decentral production with reasonable costs and low energy demand. Value added in agricultural branch is high, when selling the fuel directly to the user. Furthermore, rapeseed oil fuel has a high biodegradability and a low ecotoxicity, which makes it not hazardous to waters.

Rapeseed is the most important oil crop in Germany, because cultivation of rapeseed is well established in agricultural practice and high oil yields from 1.2 to 1.6 tons per hectare is achieved. Rapeseed oil can be produced in small agricultural production units (decentralised oil mills) and used for many different applications, such as edible oil, lubrication agents or fuel for adapted engines. Important fuel properties, such as cold flow properties and oxidation stability are more favourable for rapeseed oil, than for other plant oils.

Growing winter rapeseed has been extended during the last years and reached an area of about 1.3 million hectares (table 1). The total yield increased to 5 million tons, which is a plus of 23 % compared to the average yield of the last 5 years.

Table 2: Growing and yield of winter rapeseed in Germany

	Unit	2000-2004	2005	Increase
Growing area	1,000 ha	1,184.7	1,323.1	11,7 %
Yield per hectare	tons/ha	3.42	3.78	10,5 %
Total yield	1,000 tons	4,048.7	5,005.0	23.6 %

The presently high demand on rapeseed oil fuel, used in adapted automotive and stationary diesel engines is caused by increasing prices for fossil diesel fuel and heating oil. Fossil fuel prices are high, because of a worldwide increasing demand on fuels with a simultaneous decreasing supply of resources. Biodiesel prices usually follow diesel fuel prices, whereas rapeseed oil fuel prices are connected to the less fluctuating world market prices for plant oils (figure 1).

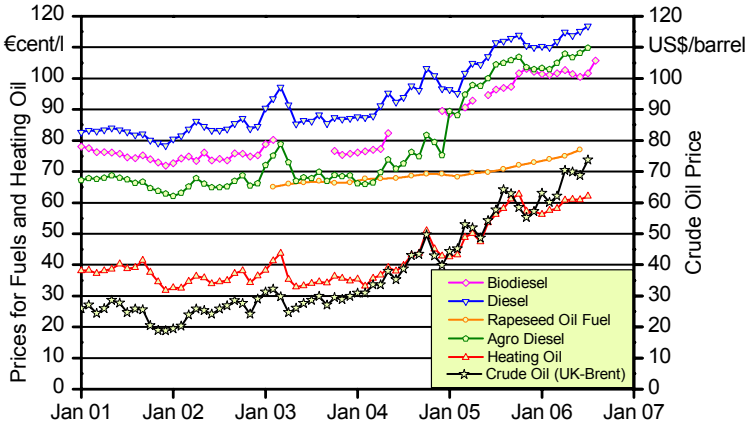

Figure 1: Price development of crude oil and fuels

Apart from the higher prices for fossil fuels, advantageous regulations for biofuels are another reason for their positive development in Germany. The EU directive 2003/30/EC on the promotion of the use of biofuels or other renewable fuels for transport (minimum proportion of biofuels by 31st of December 2005: 2 %, by 2010: 5.75 %) and the council directive 2003/96/EC of the 27th of October 2003 on restructuring the community framework for the taxation of energy products and electricity, which enables excise tax reduction on biofuels are building the frame.

The new German "Energy tax law" assures a tax exemption for rapeseed oil fuel until 31.12.2007. Afterwards the fiscal privilege for rapeseed oil fuel will be reduced gradually. Starting from 2008 the tax rate amounts to 0.10 €/litre and will be increased subsequently until 2012 to 0.45 €/litre. Supplementary to these tax rates, vegetable oils which are used as pure biofuels in agriculture and forestry, remain completely exempted from tax. This tax exemption is durably and without any limitation of quantity. Together with a new regulation for agro-diesel fuel, which causes rising fuel costs for farmers, a strong demand for rapeseed oil compatible tractors and machinery can be observed.

2. Production of Rapeseed oil: Today 13 industrial and more than 300 decentralised oil mills produce rapeseed oil, mainly for energetic use in Germany. Processing in industrial oil mills is done by "warm pressing" and following solvent extraction, which leads to high oil yields of up to 99 %, but on the other hand requires several steps of refining. Due to the high expenditure, only plants with great production capacities of up to 3,000 tons oilseed per day operate economically successful (table 2).

Table 2: Features of industrial and decentralised oil mills

	Unit	Industrial oil mills	Decentralised oil mills
Number of plants (state 2006)		13	> 300
Processing technology		"warm pressing" + solvent extraction and refining	"cold pressing"
Processing capacity	tons/day	up to 3,000	0.5 – 25
Energy consumption	GJ/ton	1.7	0.1 – 0.5
Oil yield	%	99	75 – 85
Total rapeseed oil production (2005)	1,000 tons	2,150 – 2,180	170 – 200
Consumption of rapeseed oil fuel (2005) total	1,000 tons	196	
from decentralised oil mills	1,000 tons		50 (estimated)

As for industrial plants, also for decentralised plants, oil processing technology is state of the art. Equipment is now provided by many suppliers. The production is characterised by "cold pressing" with processing capacities from 0.5 to 25 tons per day (or more) without the need of refining, with low energy consumption, but also with lower oil yields from around 75 % to 85 %. Despite plenty of experiences, process optimisation is still possible and necessary for both, high production efficiency and high product quality. Furthermore, an effective quality management system for rapeseed oil fuel has to be established.

Table 3: Pre-standard DIN V 51605 (selected data), [1]

Properties / Contents	Unit	Limiting value	
		min	max
Characteristic properties for rapeseed oil			
Density, 15°C	kg/m³	900	930
Flash point	°C	220	
Heating value	kJ/kg	36,000	
Kinematic viscosity, 40°C	mm²/s		36
Carbon residue	mass-%		0.40
Iodine number	g/100g	95	125
Sulphur content	mg/g		10
Variable properties			
Contamination	mg/kg		24
Acid value	mgKOH/g		2.0
Oxidation stability, 110°C	h	6	
Phosphorus content	mg/g		12
Ash content	mass-%		0.01
Ca + Mg content	mg/kg		20
Water content	mass-%		0.075
Ignition performance		39	

3. Demands on Rapeseed Oil Fuel Quality: The demands on rape seed oil fuel quality were first defined in the Quality Standard for Rapeseed Oil as a Fuel (05/2000), which was the basis for the new developed pre-standard DIN V 51605. Therein the latest cognitions, regarding rapeseed oil production, analytics and engine applications are incorporated (table 3).

Beside others, like ignition performance, the main difference between rapeseed oil and diesel fuel is the viscosity. Whereas the viscosities of both fuels are similar at high temperatures, viscosity from rapeseed oil increases superproportional with decreasing temperatures. This is the reason, why conventional diesel engines have to be adapted to the special needs of rapeseed oil fuel, before rapeseed oil can be used properly.

4. Rapeseed Oil fuelled engines [2]: Purpose built rapeseed oil fuelled engines of recent times were developed in the 1980s. The most famous designer was Ludwig Elsbett. Because of the fast ongoing engine development to fulfil the demands of the user and the emission regulations, today adaptation of conventional diesel engines is typical. Structural measures for adaptation can be (among others):

- Preheating of the fuel
- Exchange of fuel and injection system components
- Modification of injection pressure and time
- Modification of the combustion chamber
- Adaptation of the engine management system
- Additional diesel fuel system (for cold start and low load operation).

In Germany about 50 mainly small sized enterprises offer solutions for the technical adaptation of conventional diesel engines for the use of pure rapeseed oil. Adaptation costs vary from 1.000 to 7.000 €, depending on adaptation technology and engine size. Adapted engines are used in cars, busses, trucks, tractors, agricultural and construction machinery, stationary engines, trains and boats.

Up to now in Germany far more than 12 000 vehicles and stationary engines are operated with pure rapeseed oil fuel (approximately 50 % cars and 50 % trucks, tractors and others). Operational reliability is high for many engine types and most of the adaptation technique.

Economic efficiency is best for high engine utilisation, high price difference between diesel and rapeseed oil fuel, as well as high fuel consumption.

Within a field survey 111 rapeseed oil fuelled tractors of 10 different manufacturers and 7 adaptation companies are being tested for their technical and economic feasibility in practical use for three years. Scientific monitoring is done by the University of Rostock. Promising first results encourage the agricultural machinery industry to consider new developments of rapeseed oil fuelled tractors. Results are:

- Tractor malfunction depends on tractor and engine type as well as adaptation measures
- Functional efficiency is best for Deutz engines in Fendt and Deutz-Fahr tractors (50 of 66 tractors showed no or only marginal failures)
- Engine performance, limited exhaust gas emissions and fuel consumption is similar to diesel fuelled tractors.

Prospects

Favourable general framework, which resulted in high price differences between diesel and rapeseed oil fuel led to an unprecedented demand on rapeseed oil production facilities, rapeseed oil fuel and rapeseed oil fuelled engines in Germany. Oil

mill capacities are being extended, a significant scale has been reached already with locally high densities of oil mills.

For further development of rapeseed oil fuel utilisation with all advantages for the environment and regional economy, existing framework conditions, especially tax exemption or moderate taxation of biofuels, need to be continued. Existing insecurities, like biofuels taxation beyond 2007 or ambiguous regulations in the Renewable Energy Sources Act (what kind of plant oil and which processing technology is allowed for full power feed-in proceeds) need to be cleared, to allow better planning security for investors.

The present bottleneck in rapeseed oil supply needs to be removed by higher rapeseed acreage, more rapeseed imports and enlargement of plant capacities. Enlargement of production capacities can be rewarding, however, locally high densities of oil mills can cause competition about customers for oil and press-cake. Thus, despite the high efficiency of oil mills today, it is advisable to focus on local markets. Otherwise a decreasing demand, e.g. due to higher taxation of biofuels or the use of other plant oils for biodiesel production, could suddenly lead to poor plant utilisation.

Quality demands for rapeseed oil fuel are widely known and defined in the new pre-standard DIN 51605, which will be introduced in practice, soon. Quality assurance systems for decentral processed rapeseed oil are being developed and established.

Although rapeseed oil fuel producers and users are mainly aware of the need of quality assurance, only few act sufficiently. Thus, further consulting is necessary and additionally measures for quality improvement and assurance need to be enforced for better engine operation reliability and fulfilment of emission demands.

Engine adaptation technique is available and improved. Companies for adaptation arise and have full order books. The demand is especially high for agricultural machines, due increased agro-diesel costs and the possibility of agricultural fuel production.

Experiences in field tests with rapeseed oil fuelled engines are all in all better than expected. This applies for operation reliability, emissions and efficiency. The agricultural machine industry considers the chances of rapeseed oil technology and engages in the development of rapeseed oil fuel compatible tractors.

Besides further improvement of engine adaptation technique and development of the adaptation of modern engines, insecurities regarding warranty agreements for rapeseed oil fuelled engines need to be abolished. This can be solved either by close co-operations between engine adaptation garages and engine industry or new product development of the engine industry. Further tests with modern engines are necessary and exhaust gas after-treatment, like particulate filter systems have to be optimised for rapeseed oil fuel.

Prior applications for rapeseed oil fuelled engines are either fields, those are only a limited number of different engine types with high items and/or environmental sensitive areas, like tractors and agricultural machinery, transportation vehicles with high degrees of utilisation and cogeneration plants. With continuing great demand of the users, rapeseed oil fuelled tractors may soon be sold industrially with high product quality and reasonable adaptation costs.

Conclusions

The results show, that present conditions offer good prospects for a further extension of rapeseed oil fuel technology in Germany. This is mainly due to highly competitive prices of rapeseed oil fuel and a steady increasing number of contented users, demanding rapeseed oil fuelled engines. The prior applications for rapeseed oil fuel are niche markets, such as environmental sensitive areas and agricultural machines, due to a limited number of engine types and the advantage of closed economic and material flow circuits.

There is also a great demand on information about rapeseed oil fuel of both, the producers and users. Therefore a country-wide information and consulting program was initiated by the Agency of Renewable Resources (FNR). There is the challenge of clearing unsolved questions by further research. Chances are now particularly favourable for an extended use of rapeseed oil fuel with major benefits in adapted diesel engines for many applications.

References

[1] Deutsches Institut für Normung e.V. (2006): DIN V 51605: Fuels for vegetable oil compatible combustion engines - Fuel from rapeseed oil - Requirements and test methods. Beuth-Verlag GmbH (available at www.beuth.de or www.din.de).

[2] Thuneke, K. (2005): Rapsöl als Kraftstoff für die mobile und stationäre Nutzung. In: (Hrsg.) KTBL: Dezentrale Ölsaatenverarbeitung. KTBL-Schrift 427, Darmstadt, S. 51-70, ISBN 3-7843-2171-2

TRADING AND POLICIES

Outlook for development of supply/demand for oilseed and oilseed products

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Oilseeds have recently reached a significant position on the world market and become a major agricultural commodity.

The strong growth of demand for both vegetable oils for human consumption and protein-rich meals for animal feed have resulted, over the last 30 years, in a making of oilseeds one of the fastest growing agricultural commodity. An average annual rate of 3 to 4 % of growth has brought oilseed world production at around 400 million tons, almost in line with that of major grains as rice (400 million tons) or wheat (600 million tons).

Around 50% of oilseed production is traded on the world market. This situation of high export orientation is very specific to oilseeds compared to other major agricultural commodities resulting in an ever more prominent rank of oilseeds and oilseed products on the world trade. With around $50 billion of trade value, oilseeds represents twice that of all cereals ($25 bn) and meats ($22 bn); in term of volume with 180 million tons (120 meals and 60 oils) oilseed trade compares to that of cereals (200 million tons). Continuation of these trends will accentuate the rank of oilseeds on the markets.

Soybean represents 60% of oilseed production worldwide. Soybean and soybean products make up to 70% of oilseed world trade. South America (Brazil-Argentina) have developed soybean production and exports and since 2000 have overtaken the USA, the long time leader on that market. Nevertheless, Brazil recent monetary adjustments have led to economical difficulties for export driven agricultural sector. This situation, compounded with sanitary problems (soybean rust) has stopped the soybean "fever" in this country and even resulted in a reduction of soybean acreage since 2004.

Rapeseed production (12% of world production) is currently experiencing a strong growth worldwide with a production of around 50 millions tons in 2005, up from the former record of 40 million tons only 2 years ago. Current rapeseed area expansion is responding to a worldwide growing interest for energy (biodiesel) for which rapeseed oil presents an excellent technical profile.

Sunflower (8%) is developing in Russia and Ukraine, both countries having become the world first sunflower producing region.

As significant other oilseeds must also be mentioned cotton (11%) and groundnut (8%).

If soybeans remains the major oilseed for the meals, on the vegetable oil market, palm and soybean oils are the two leading oils with a production of around 33 million ton each, while rapeseed oil (16 million tons) comes 3rd, before sunflower oil (10 million tons).

Oilseeds are grown for more than half of the production in a few exporting countries : the Americas (US, Mercosur for soybean, sun– Canada for rapeseed), South East Asia (Indonesia/Malaysia for Palm oil). China, India, Europe, Australia are other significant oilseed producers.

The oilseed demand on the world market has long been driven by Europe's soybean and soybean meal imports. This is not any longer the case : Asia and especially China is now driving the trade with an ever growing share of world imports. China alone imports over 30 million tons of soybean and soybean products, and the annual 10% growth of these imports will soon take China at the first rank of for this commodity.

On the oil world market, India stays the major import market.

The outlook for future expansion of demand from traditional markets of oils and meals remains very promising. The scale of future needs is given by the gap between per capita consumption in emerging countries compared to that in developed countries (meats : 50 kg vs 100 kg, vegetable oils : 10 – 15kg vs 35kg). The current economic development taking place in emerging countries is fuelling a 4-5 % growth trends for white meats and vegetable oils markets and hence, for oilseed products.

In addition to traditional demands, the new demand for energy uses is already active on the vegetable oil market. FAME (fatty acid methyl ester) is used as a biofuel in all diesel motors in blends from 5 up to 10% and with minor modifications up to 100%.

Recently, high prices for energy have triggered a booming interest worldwide for biodiesel as well as for biofuel in general.

In Europe, where a large part of individual vehicles run on diesel, biodiesel current production of 4 million tons is planned to reach up to 12 million tons by 5 to 6 years (corresponding to the official EU objective of 5,75% of fuel for transportation).

Biodiesel production could also develop in the USA up to 2 – 3 million tons by 2012, and in Brazil up to 2 million tons

by 2013. Malaysia and Indonesia are also planning to produce up to 5 million tons.

Apart from use as biofuel, it may also be mentioned that vegetable oil -palm oil- has been used for electricity production in Europe these last 2 years.

Rapeseed currently benefits from biodiesel demand due to the technical advantages of rapeseed oil over other oils – resistance to oxidation and low solidification temperature -. This situation translates in a positive evolution of rapeseed oil prices with a significant premium over other oils prices ; as a result, areas dedicated to rapeseed has expanded in major producing countries.

In conclusion, supply/demands fundamentals offer a very positive outlook for future developments of oilseeds in general and rapeseed in particular.

International competitiveness of oil and protein crop production systems (T)

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Abstract

In an increasingly globalized world, agricultural production of the different products will be re-located to those regions and farms which exhibit the lowest cost of production. The ***agri benchmark*** cash crop network of farm economists, advisors and growers provides for a conceptual and infrastructural framework to run international comparison on a uniform basis. ***agri benchmark*** cost calculation for different oilseeds reveal strong variation in cost and marked disadvantages with regard to cost of production – primarily of (Western) European farms. The most important factors for differences in operating cost across the board – in itself the most important cost category – are farm size and production systems. Government payments and protection from international competition lead to large differences in returns as well. Therefore high cost of production for high return countries are in line with economic theory. However, it remains to be seen whether low cost oilseed alternatives will become relevant in the EU – especially in biodiesel production - and consequently create pressure on rapeseed prices as well as the need for adjustments on European oilseed growing farms.

Key words: cost of production, competitiveness of farms, production systems, typical farms, biodiesel.

JEL Codes: Q12, Q17, Q18

1. Introduction

The global increase in bioenergy production affects global oilseed production in three ways: On the one hand rapeseed is a major source for biodiesel, which is of particular relevance to Europe. On the other hand, on the farm level oilseeds have to compete with other agricultural raw products especially for ethanol, the most important biofuel globally. Last not least biodiesel can be produced from various feedstock – hence the question arises how competitive are the different oilseeds. In the long run cost of production of the relevant oilseed crops are the relevant indicator for competitiveness.

agri benchmark[1] provides for a network of agricultural economists and a number of tools to analyze these differences in cost of production on farm level. This analysis includes a thorough look on the different production systems which tend to have a major impact on cost of production.

2. *agri benchmark*: background, motivation and vision

Until the late 1980s, international farm comparisons were only carried out on an ad hoc-basis, mainly within the scope of Ph.D.-studies, some of which were performed by one of the authors of this paper [1, 2].

The results of these comparisons were recognized as being useful. At the same time they revealed the following problems:

- required data was either not available at all or not available in the scope required to perform total cost analysis
- available data did not allow costs to be differentiated into their price and their volume components to explain cost differences
- available data was usually not comparable across countries

In order to overcome the problems of the past as outlined above in 1997 the International Farm Comparison Network was founded which later became ***agri benchmark***. The vision of ***agri benchmark*** is 'to improve understanding of farming world-wide'.

agri benchmark is a world-wide association of agricultural scientists, advisors and farmers. Within the framework of this co-operation, farms and agricultural production systems are defined that are typical for their region. For the most important agricultural products and regions in the world, ***agri benchmark*** delivers answers to the following questions:

- How is farming done (farming systems, production technology)?
- What is the level of variable and total production cost?
- What are the reasons for advantages and disadvantages in cost of production?
- What is the perspective of agricultural production at the locations considered?

In this way, ***agri benchmark*** can be seen as a **navigation system** in the rapidly changing global agricultural sector.

The Cash Crop branch of ***agri benchmark*** was established in 2004, until now it is active in 13 countries – including

[1] *agri benchmark* is a global network of farm economists working in the branches Cash Crops, Beef and Dairy; the project is jointly managed by DLG and FAL. For further detail see www.agribenchmark.org.

countries like Brazil, Argentina, US and Canada or Ukraine. The group of international partners meets once a year in a conference to discuss results, to prepare Cash Crop Reports [3] and to decide on future projects of the network.

3. Global trends in oilseed and rapeseed markets

For a better understanding of the global oilseed market, the interaction between the different oilseed crops as well as the global trade some key figures are presented here.

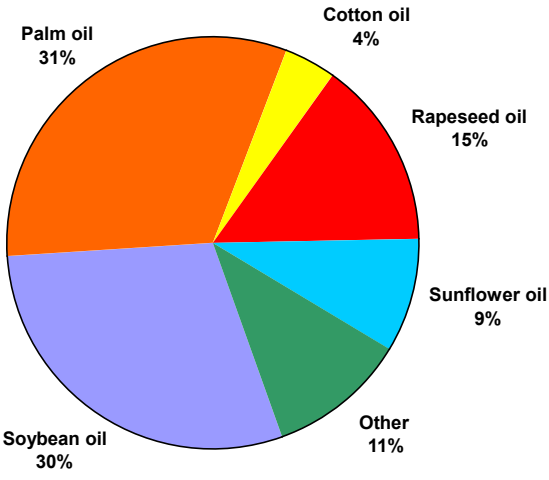

Figure 1: Global market shares of different plant oils

Figure 1 reveals that next to soybean and palm oil which are by far the most important plant oils, rapeseed oil is the #3 with a market share of 15 %. Since 1999 this pattern of market share was essentially stable, even though we have had an increase in global oil production by 40 %.

In all major uses the oils mentioned here are close substitutes, therefore it has to be assumed that prices for soybeans and palm oil respectively determine global plant oil prices.

Source: Oil world (2006), own calculations

However, as displayed in figure 2, since 2005 there has been an ongoing trend towards a stronger increase in rapeseed oil prices relative to soybean oil.

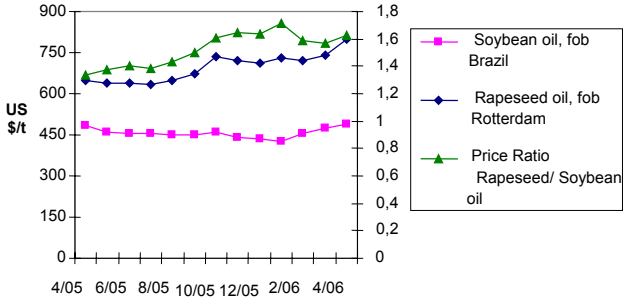

Figure 2: Evolution of soybean and rapeseed oil prices

This increase in rapeseed oil prices relative to soybean prices is primarily driven by the demand of biodiesel factories for rapeseed oil, which is almost the exclusive feedstock for European biodiesel production. Legally defined technical standards are the cause for this dominance of rapeseed oil. In 2006 about 50 % of the European rapeseed production was converted into biodiesel.

Source: USDA (2006), own calculations

Just recently the EU Commission issued a paper on biofuels [4] in which they called for lesser stringent technical standards for biodiesel which in turn might open the market for non-rapeseed oils. With regard to future development it has to be kept in mind that the three major producers of soybeans Brazil, the US and Argentina are increasingly producing biodiesel based on soybean oil.

The increase in rapeseed oil prices not only triggered increasing imports of oil but a significant increase in European rapeseed production well above the global growth. Europe now is the most important rapeseed producer; back in the 1980th China was the largest. All other major producing countries maintained their share in global output during this period even though global production increased by 200 %.

4. *agri benchmark* farm level data

The competitive position of oilseed crops can be evaluated with the help of some selected ***agri benchmark*** results.

At the first glance farm data on total **cost of production** make us believe that in a more globalized agriculture there is little room for high cost producers – primarily located in Europe. Their costs of production are currently significantly higher than those of overseas producers, especially those from South America (see figure 3). The gap between the five most expensive producers and the five least cost growers is more than 200 $/t RE[2] or 175 % respectively.

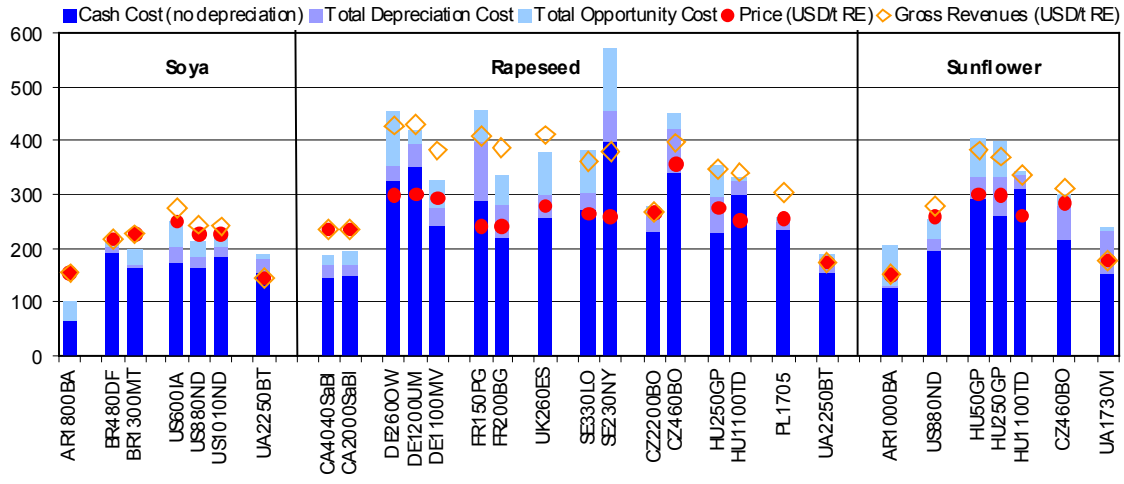

Figure 3: International cost comparison for oilseed production (USD/t RE)

Source: Zimmer et al. (2006)

Figure 3 also reveals significant differences in cost of production within the group of rapeseed producers: The two Canadian farms spend less than 200 $/t RE whereas two German, one French and the one British farm realize total cost of more than 400 $/t RE.

The disadvantage in cost of production is primarily driven by **operating cost**: On average almost 40 % of total costs are operating cost; they range from about 60 % down to little above 20 %. Regression reveals that over 60 % of variation in total cost of production can be explained by differences in operating cost.

Operating cost themselves are driven by the **number of operations** like spraying or cultivation carried out by the grower.

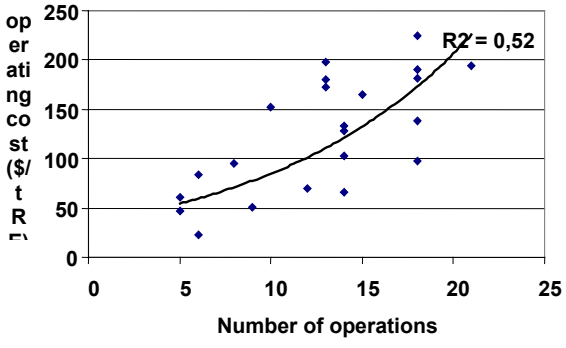

Figure 4: Number of operations: driving factor for operating cost

Production systems differ significantly between different regions and farms. In oilseed production for instance the number of operations can be as low as 5 in Argentina or as high as 21 in Germany. The impact of numbers of operations on operating cost can be analyzed with the help of a regression. As can be seen in figure 4, over 50 % of the variation in operating cost can be explained by the number of operations. The increase in yield and hence in revenue associated with more operations is not able to compensate for the increase in operating cost caused by more operations.

Source: Zimmer et al. (2006)

Due to different market prices and government payments there are **major differences in gross revenues** (see amber diamonds in figure 3), therefore cost of production are just one side of the coin. In order to take into account these differences an **analysis of economic productivity** has been carried out. The revenues per Dollar spent on operating cost are much more even across the sample than cost or revenues are. The majority of ***agri benchmark*** farms generates 2 to 3.5 $ revenue with every Dollar spent on operating cost. However, when deducting government payments from gross revenues of EU-farms most of them realize a productivity of operating cost of only 1 to 2 $.

Keeping in mind the fact that in cash crops we just started the total cost analysis and pending methodological issues like value of land have to be solved yet, these results lead to the following preliminary **conclusions**:

[2] For a comparision of different oilseed crops, a synthetic unit „Rapeseed Equivalent" (RE) has been calculated. This value is derived by the processed value of each crop relative to rapeseed taking into account different contents and values of oil and meal. For sunflower this factor is 1.034 whereas for soybeans it is 0.861. This means soybean yields expressed in RE are a bit less than originally because the accumulated value of their content is higher than the one for rapeseed.

- In oilseeds there are currently major cost advantages for overseas producers and the Ukraine relative to their EU counterparts. With the exception of Canada this implies a competitive edge of non-rapeseed oilseeds over rapeseed.
- Current high rapeseed prices relative to soybeans have widened the gap between high European gross revenues and relatively low levels for western hemisphere producers.
- High cost of production per unit of output are to a large extend driven by differences in production systems when expressed in the number of operations.

The EU is considering a mandatory blending target of 5.75 % biodiesel – even a quota of 10 % has been suggested. Due to legally defined technical standards this strategy would be primarily based on rapeseed. Therefore it seems to be likely that in future high rapeseed prices will prevail. On the other hand the low cost of production of the Ukrainian and the Canadian farms create a rather strong incentive for increasing exports to the EU and hence – depending on the volumes traded – pressure on EU price levels. This pressure will even increase to the extent future European biodiesel production will be based on cheaper non-rapeseed feedstock as suggested by the EU commission. This in turn would create the need for growers to adjust their farming systems to new price / cost ratios.

References

1 Isermeyer F. (1988), Produktionsstrukturen, Produktionskosten und Wettbewerbsstellung der Milcherzeugung in Nordamerika, Neuseeland und der EG. Kiel: Wissenschaftsverlag Vauk, Arbeit aus dem Institut für Agrarökonomie der Georg-August-Universität Göttingen [Dissertation]

2 Deblitz, C. (1994), Internationaler Vergleich von Systemen extensiver tiergebundener Grünlandnutzung: Produktionstechnische und ökonomische Analyse, Wettbewerbsfähigkeit, internationale Übertragbarkeit. Kiel: Wissenschaftsverlag Vauk, Arbeit aus dem Institut für Agrarökonomie der Georg-August-Universität Göttingen [Dissertation]

3 Zimmer, Y. et al. (2006), *agri benchmark* cash crop report 2006, Braunschweig

4 An EU strategy for biofuels, COM (2006) 34 final, p. 13

Market development and competitive forces in emerging markets of rapeseed and oilproducts

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Abstract

- Production and trade of oilseeds and its products has been experienced a tremendous increase in the last years. Serious forecast expect two scenarios:
- Production, trade, and consumption will still increase;
- Consumer demand patterns will be more differentiated, market segmentation and fragmentation will have more influence on the specification of the varieties of oleic acids and the protein fraction of oilseeds.
- These changing quality variations will have a number of interesting implications on the market driving forces especially in the various rapeseed market segments:
- Competitiveness of single oilseed varieties depends not only on price alone but a growing concern has to be put on quality characteristics.
- Because of rapeseed and its oil and protein components are exposed to a growing competition the driving market forces have to be analysed carefully in order to discover alternative and new ways of marketing.

With new varieties, market segmentation, and volatility in markets we want to demonstrate that the way in which marketing, trade, and consumption are being practiced is changing. Additional, we analyse the changing role of consumption patterns. Marketing and trade has changed or is about to change in a way that the relations within the food and non-food supply chains are more and more end-consumer driven. Customers sought not just simple transactions, but wanted relationships, networks, and interactions. The food and non-food supply chain of rapeseed and its oil and protein components will provide an interesting focus for study. We will present first empirical results of an ongoing research work on the driving market forces, the competitive networks and trade relations within various evolving market segments of the food and non-food use of rapeseed and its rival oilseed products in the German and some European markets. Decisive for the international competitiveness of the rapeseed breeding industry and its relations to the oil and protein processing industry is the successful integration of customer requirements (their needs and wants).

1. Introduction

Both, growing and trading of oil seeds and its derivatives, have experienced a large expansion worldwide in the last few years. Especially the increasing incomes together with a growing population in Asia have lead to high increase in the demand of vegetable oils and meal. In addition to that there are worldwide efforts to build up and to extend bio diesel manufacturing capacities (Shwedel et al., 2005). The above-mentioned factors will significantly influence the development of the market in the next years and cause a continuation of the positive trend (OECD/FAQ, 2006, USDA ERS, 2006). The growing markets in Europe and Germany mainly result from the demand in the bio fuel sector due to subsidization in most countries. Contrary to that the growth in demand for edible oil and oil seed meal is considerably slower. In Germany e.g. stagnation in consumption of edible oils and oilseed meals is prevalent (ZMP, 2006). Due to the given possibility to substitute several oils and flours and due to their unproblematic transport, in Germany the domestic resources rapeseed and sun flowers compete with products from Soya or palm oil that are substantially available on the global market. Therefore, under these conditions we will think about how to improve the competitiveness of products from domestic oilseeds in order to create competitive advantages. We are doing this by analyzing how far the value-chain of oil seed production and processing have to meet the consumers´ demand in order to improve consumption and which conditions for a successful cooperation between the involved actors are necessary.

2. Market development and market structure

Table 1 shows the development of the market for vegetable oils and fats for Germany in recent years. With a production of totally 3,16 million t. vegetable oil domestic oil mills in 2005 for the first time processed more than 3 million t. Rapeseed oil included. Also the domestic use reaches with 3,79 million t. a new record level. This development exclusively has to be atrributed to the growth in the bio fuel sector (increase of about 100%) (UFOP 2006).

The future evolution of the oilseed markets heavily depends on the development of the commodity marketing system as the driving force. Two primary distribution systems exist for oilseeds and its derivatives in commercial agricultural systems. One distribution system is focused on commodity crops (like bio-diesel), where the emphasis is on homogeneity. The other distribution system is focussed on high-value traits, but has been utilized primarily for small volumes.

Homogeneity is a fundamental attribute that has permeated the traditional oilseed supply chain. In the commodity value chain, farmers produce generic oilseed crops, all of which are viewed identical, despite the fact that they were produced by a

large number of different peasants. After harvest, farmers deliver their oilseeds to a first-handler. The first-handler receiving the crops is not interested in differentiating these oilseeds for different end-uses, but is interested in blending oilseeds to meet physical limits for certain grades in outbound shipments.

Table 1: Market Development for Oils and Fats (in 1,000 t)

	2000	2001	2002	2003	2004	2005
Production	2,830	2,829	2,813	2,752	2,873	3,160
Imports	1,769	1,940	2,132	2,180	2,296	na
Exports	2,012	2,222	2,296	1,872	1,801	na
Domestic use	2,549	2,563	2,689	3,061	3,383	3,878
Feedstuffs	134	400	405	411	415	na
Industrial Use	714	626	750	1,158	1,576	na
Food processing use	522	524	505	438	420	na
Food consumption	1,179	1,013	1,029	1,054	972	na

Source: BMVEL, various issues; UFOP, 2006

This commodity orientation has important implications. First the capability to coordinate a large and diverse sector such as the oilseed chain with minimal information flow throughout the sector has been a major strenght of the sector. Although commoditiy output meets the general specifications of the customer at the next level, it may not optimally meet the specific needs of any one customer. The associated loss of efficiency at the customer level is offset by the considerable flexibility of supply offered by the commodity system and its low cost. This is one reason that commodity agriculture has been successful. However, one side effect of this structure is that knowledge creation tends to be concentrated within each segment in the chain rather than disseminated throughout the chain.

The traditional supply chain for oilseeds, emphasizing homogeneity, has been in place since the production of oilseeds began in earliest 1970s. An alternate supply chain exist in parallel to the homogeneous commodity market and is used for some differentiation of oilseeds, particular in markets for human consumption and sophisticated industrial use. There are many forces, which independently and combined, are putting pressure on the traditional production and marketing practices in agriculture. Increasing consumer sophistication, technological change, competition, environmental concerns, and biotechnology are some of the factors that are influencing today´s agricultural marketplace.

Consumer sophistication has resulted in interest in foods that go beyond traditional concerns of price and presentation. The enhanced consumer interest primarily involves three aspects if foods they eat: food safety, health isues, and perseptions associated with particular production practices. Consumers also have expressed interest in foods that improve their health, such as products with lower cholesterol or which may reduce their risk of certain cancers. The ability to supply either the attributes that provide the health or nutritional component, or provide the information on production practices, requires an identity-preserved market channel. Consequently, the analysis of the supply chain for oilseeds has to start at the traditional end on the stage of the consumption.

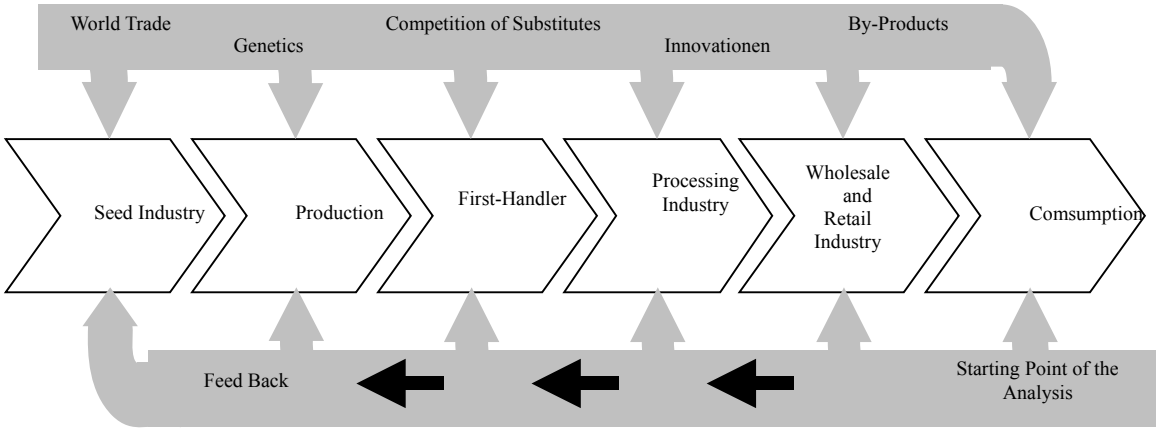

Figure 1: Supply Chain for Oilseeds and its Derivatives

Figure 1 shows the approach to the considerations. The central starting point of the investigation is the assessment of the user and consumer needs in the particular market segments of the food and non-food sector. The different market segments were observed regarding their present development and trends arising from it were derived. These qualitative and quantitative trend developments arising on the side of demand are in turn estimated regarding their repercussions on the whole oil seed chain and the involved actors working in it. In addition to the repercussions from the side of sales the effects of the global environment (socio-cultural, technological, political, ecological) as well as of the competitive environment (competition, industry) were involved. These impacts on the value-added chain are shown by the arrows above the value-added chain.

3. Competitiveness of domestic oil seeds

While hitherto oil seeds with differences only in the agronomic parameters as grain harvest, oil harvest or harvest stability were processed along the supply chain, now it has been increasingly switched over to focussing on special quality parameters in order to create a differentiation advantage. The quality parameters relevant for a product differentiation are modified fatty acid patterns, a modified amino acid composition of the oil meal as well as the accumulaton with secondary plant ingredients (Leckband & Voss, 2001). However, in the following only the modification of the quality parameter fatty acid composition will be investigated.

The domestic oil seeds rapeseed and sun flowers show in their natural fatty acid patterns a comparably high long chain monounsaturated fatty acid (LCMUFA) and long chain polyunsaturated fatty acids (LCPUFA) content. Thus, they compete with palm oil, olive oil, and soybean oil. The introduction of domestic oil plants with new quality characteristics has to be in competition to alternatively usable raw materials linked with a balanced use for all involved actors of the value-added chain. Figure 2 shows the conditions for a successful commercialization of domestic oil plants with new quality characteristics. The most important condition is the acceptance of this new product by the consumer. The consumer has to be able to accept the product as of higher value and to realize a personal additional benefit from the new product. As soon as the consumer demand has been realized also the processing industry will have an increased interest in obtaining the new raw materials in order to extent the existing range of products and thus to open new markets.

The First-Handler and the oil mills will only meet the demands of the industry for the supply with the new raw materials, if the consumers pay for the additionally costs caused by handling the new raw materials' quality through separating from the conventional qualities at processing, storage and transport (Darroch et al., 2002). But the farmer will only cultivate the new product qualities demanded by the industry if he can get assured producer prices and purchase quantities to optimize his profit by contract cultivating (Jefferson-Moore & Trexler, 20059.

The seed breeding companies eventually want to get compensation for the added value of the new breeds in terms of the breeder license. Furthermore the supply with a breed achieving the quality demanded by the industry is connected with obtaining a competitive advantage over the rival businesses (Leckband & Voss, 2001).

However, there is often a lack of fulfilling one or more conditions for the introduction of new qualities. Thus, many consumers doubt the benefits of plants with genetically modified fatty acid pattern (Moon & Balasubramanian, 2003) or the announced higher added value is critically assessed by the market partners (Darroch et al., 2002).

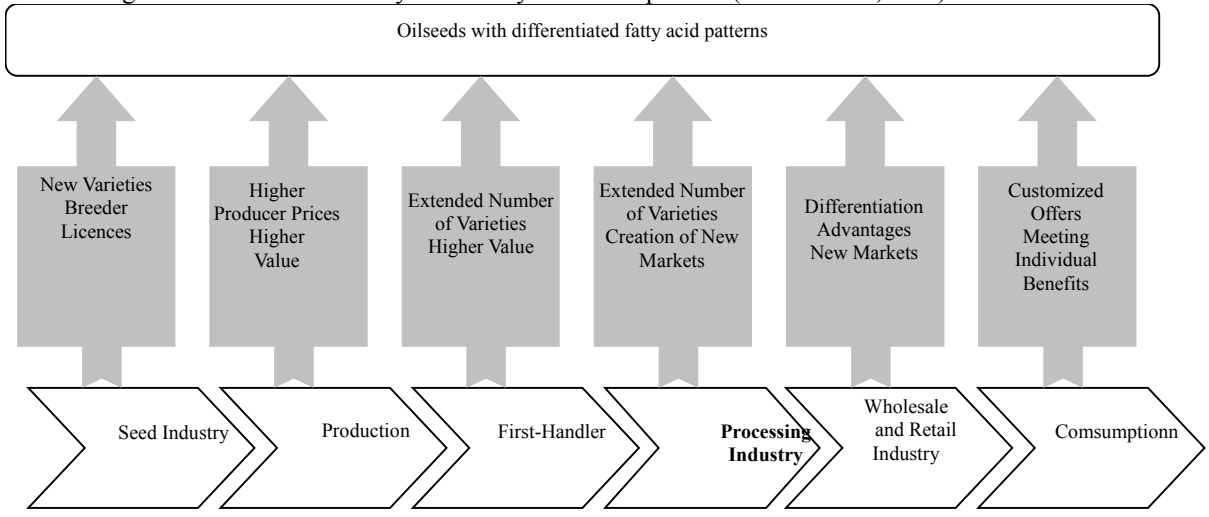

Figure 2: Necessary Conditions for Successful Oilseed Differentiation

The criteria applied by every phase of the value-added chain to domestic oil seeds with modified fatty acid pattern accordingly have to be considered also in the development of new breeds. Thereby, the underlying criteria can be very complex.

Fulfilling these criteria requires an intensive communication between the processing and the seed industry in order to avoid potential differences in starting-phase. An example for such divergent cooperation activities the processing of rapeseed rich in lauric acid. As from the industry's point of view the criteria preparation and processing and the involving criteria price are not fulfilled, the rapeseed rich in lauric acid has not yet succeeded (Sauter/Hüsing, 2005). In contrast, the so called high oleic (HO) oil seeds can be seen as a successful product introduction. They include the breeds rich in high oil acid like sun flowers, rapeseed and Soya having been able to enlarge their share in the world production of the particular oil plants and according to estimates also being able to increasingly enlarge it (Kleingartner, 2002, p. 3; Frauen, 2004; soyatech, 2006).

In the case of HO-sun flowers and HO-rapeseed it can be assumed that in Germany the production increase of these both breeds will be larger than the increases of the particular oilseed. The reason for the success of these oilseeds is not only a result of fulfilling the described criteria and conditions, as the high oleic breeds show a wide spectrum of use both in the food and non-food sector. Because of the multitude of potential consumer the oil mills can gain large batches of these oilseeds and compensate the additional cost for the separate handling of these oils by the generated mark-up. The same applies to the

First-Handler. Especially in customer contractual relations the farmers don't run the risk of not disposing their raw materials. At the same time they will benefit from the mark-ups of the consumers if they compensate possible harvest losses associated with the new breed. The seed breeding companies in turn can catch up with the breeding progress by setting higher seed prices,

Which conclusions regarding development potentials in specific market segments can be drawn on the basis of the analysis? This question will be answered with the development of rapeseed oil in the market segment edible oils (mostly private household consumption). Currently, 9 per cent (173,000 t) of the total edible oil demand by the private households results from rapeseed oil. Since the middle of the 90's this segment has a steady incresse. Numerous efforts of all links of the value-added chain emphasizing the benefits of its nutritional physiological characteristics like a close ω3/ω-6-relation can be viewed as a result to support that product (UFOP, 2006). At the same time the oil mills tried to invest in the improvement of the product qualities (Matthäus & Brühl, 2003). These efforts have helped rapeseed oil to an extremely positive image. In the meantime rapeseed oil can be found in all quality and price segments. Besides the increasingly offered mixtures with other oils an additional growth potential is arising by the emergence of high-oleic special oils for the use in cuisine. In perspective, additional market potential is emerging by the supply of rapeseed oil of genetically modified plants providing the consumer with an additional benefit in terms of ingredients with a high nutritional value (Heinz, 2006).

4. Concluding remarks

The present analysis has shown, that the efforts launching new oilseeds with a modified fatty acid pattern will mainly be successful if they cover a wide spectrum of use and at the same time fulfil some conditions and criteria demanded by the particular actors of the value-added chain. This requires a high degree of networked activities of the involved actors. The global environmental factors like the influence of politics or emerging technical innovations being able to influence market development as the examples genetic engineering or bio fuel have shown, also have to be considered. Finally, for an increased establishment of oilseeds with specific fatty acid patterns the formation of vertical and horizontal alliances and co-operations has to be extended.

6. References

BMVEL (Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft) (ed.) (various issues): Statistical Yearbook of Nutrition, Agriculture, Forestry, Münster-Hiltrup.

Darroch, M. A.; Akridge, J. T. und Boehlje, M. D. (2002): Capturing value in the supply chain: the case of high oleic acid soybeans. In: International Food and Agribusiness Management Rev., No. 5, S. 87-103.

Frauen, M. (2004): Einsatz von Gentechnik bei Raps. Präsentation anlässlich des UFOP-Gesprächs mit dem Verband der Margarine-Industrie vom 20. 10. 2004 in Hamburg.

Heinz, E. (2006): First breakthroughs in sustainable production of "oceanic fatty acids". In: European Journal of Lipid Science Technology, Band 108, S. 1-3.

Kleingartner, L.W. (2002): NuSun Sunflower Oil: Redirection of an Industry. In: Janick, J. und Whipkey A. (Hrsg.) (2002): Trends in new crops and new uses. Alexandria, ASHS Press, S. 135-138.

Leckband, G. und Voss, A. (2001): Raps als Bioreaktor für Nachwachsende Rohstoffe in der chemischen Industrie. In: FNR (2001): Nachwachsende Rohstoffe für die Chemie – 7. Symposium 2001. Landwirtschaftsverlag, Münster-Hiltrup.

Matthäus, B. und Brühl, L. (2003): Quality of cold-pressed edible rapeseed oil in Germany. In: Food, Vol. 47, Nr. 6, S. 413-419.

Moon, W. und Balasubramanian, S. K. (2003): Is there a Market for Genetically Modified Foods in Europe? Contingent Valuation of GM and Non-GM Breakfast Cereals in the United Kingdom. In: AgBioForum, Band 6, Heft 3, S. 128-133.

OECD/FAO (Hrsg.) (2006): OECD-FAO Agricultural Outlook 2006-2015. Paris.

Sauter, A. und Hüsing, B. (2005): TA-Projekt Grüne Gentechnik – Transgene Pflanzen der 2. und 3. Generation. Endbericht. TAB Arbeitsbericht Nr. 104. Berlin.

Shwedel, K.; Reca, A. und Scaff, R. (2005): The Oilseed Industrie: Surviving in a Changing Competitive Environment. Rabobank International F&A Research and Advisory.

UFOP (Union zur Förderung von Öl- und Proteinpflanzen) (Hrsg.) (2006): Report 2005/2006. Berlin.

USDA ERS (Economic Research Service) (2006): Agricultural Baseline Projections: Global Agricultural Trade, 2006-2015. Briefing Rooms, Washington/USA.

ZMP (Zentrale Markt- und Preisberichtsstelle) (Hrsg.) (various issues): Marktbilanz Getreide Ölsaaten Futtermittel. Bonn.

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Some topics of EU discussion on world free trade with vegetable oils from viewpoint of czech union of oilseeds growers and processors

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Abstract

WTO-rules and the debates on liberalisation of markets versus local development are forming following question:

What is good for producers, and processors of rapeseed?

What is good for consumers of rape oil?

Two subjects are tackled in more detail: Real purchase power of consumers and sustainability of producers, and processors in different countries producing rape seed oil is used to show proposals of different parties involved in the problem solution. High purchase power of EU inhabitants will increase prices of oil for Asian consumers, and can damage EU rape seed producers, and processors. It is possible to feel a fear in internal European discussions that Europe doesn't succeed to produce under competition of imports. A price increase of energy and oils can change the situation. But, recent crude oil price decrease in second half of 2006 year proves the volatility of the market. Europe is importing vegetable oil for both bio-fuels, and food already now. The question is under which conditions and measures European oil seed producers, and processors survive? Many different proposals are presented. Industrial relations in connection with combustion technology of rape oil in engines can become a solution for exodus from rural areas as a consequence of global market volatility.

Key words: Oilseed, trade, development, price, food, bio-fuels, proposals, experiences, transition.

Overview of Proposals Preventing Market Distortions and Local Development

Opinions of leaders of liberal tendencies and their opponents is discussed in next paragraphs.

General Approach of WTO Towards Agriculture

The Uruguay Round Agreement on Agriculture (URAA, 1995) defined three categories of support: *market access, export refunds* and, *domestic support*. In the first two categories the agreement targeted at the restriction of open use of means that inhibit trade, in the third category at a decrease of trade distortion caused by domestic support. The biggest result of the GATT/WTO agreement was that the tariff system became the base for agricultural trade among WTO members.

Legal, Trade, and Plan Based Solutions

Three approaches the legal, trade, and plan based solutions of global market distortions are discussed further in this paragraph.

CPE (Coordination Paysanne Européenne), Via Campesina, and their many allies in the populations are continuously working to make food sovereignty an essential right. This is considered to be legalistic approach. Agricultural policies based on food sovereignty is our solution against the dogma of 'free'-trade, and one important answer in the fight against poverty, starvation and forced migration" says CPE, and Via Campesina. Respect for this legal claim seems to be even lower then efforts to remove barriers of trade by WTO.

Australian, and Canadian free trade based statements are contradictory to the one of CPE:

Australian statement: Myths and realities of agricultural protection	
Support is needed to	*Response*
Provide for self-sufficiency	Biggest subsidiser of all – the EU – is more than 100 % self-sufficient. The EU is, e.g., the world's second largest exporter of sugar.
Keep people in rural areas	At one point Europe lost one farmer for every minute over 20 years. The average age of farmers in subsidised countries is not different to that in countries with low protection of farms.
Preserve the countryside	Fragile land has been returned to native vegetation when subsidies removed. Better to subsidise hedgerows and maintenance of 'small green fields' rather than milk production. First best policy is a direct subsidy to preserve countryside combined with free trade.
Preserve jobs	Assistance for one job is a tax on another – for every job saved there is a job lost.
Provide adequate farm income	Hasn't worked. Many farmers in the most protected markets still struggle. About 70% of EU subsidies go to the 30% of biggest farmers.
Preserve the environment	But it has been found that 80% of subsidies are perverse – they harm both the economy and the environment.
Ensure food safety	Hygiene standards are higher in New Zealand, which receives no farm support, than in protected markets. Some of the worst food safety scares have occurred in the most protected markets.

Canadian statement:

The response of some traditional agricultural powers - particularly the U.S. and the European Union - has been to protect their farmers by introducing subsidies that shield them from lower commodity prices while encouraging production to remain high. Clearly, this approach is unfair and harmful, not only to our producers, but to those farmers in the developing world who have difficulty exporting their products even as their markets are flooded with cheap U.S. and European Union products."

IFAP (International Federation of Agricultural Producers) in a letter from 12 July 2004 sent to the Ambassador Tim Groser, Chair of the WTO Committee on Agriculture Special Sessions, the IFAP stressed that it would be judging the adequacy of the WTO agricultural modalities paper on whether it met six critical objectives for farmers. These objectives are:

1. An improvement in world agricultural trade that brings real benefits to all farmers.
2. Significant progress and balanced commitments over all three pillars.
3. Sufficient flexibility in the modalities framework allowing countries to use the most appropriate instruments according to their specific national circumstances to meet agreed, measurable and equitable outcomes
4. Space for farmers to receive domestic support, so long as that support has no, or at most minimal distorting effects on production and trade.
5. Improvements in market access for all farmers, in particular those in developing countries and Least-Developed Countries.
6. Due prominence and recognition of the broad role that agriculture plays in many countries, ensuring not only food production but also many other function, including the sustainability of rural areas and environmental protection."

Eco-social Forum Europe

In an "Eco-social Forum Europe" the former Austrian vice-chancellor Josef Riegler and the German professor in ordinary, Franz J. Radermacher touched the following corner pillars of a world-wide strategy against economic speculation and social distortion:

An eco-social market economy as propagated by the "Global Marshallplan" must pursue a world-wide principle of coherence and just as much a world-wide principle of social cohesion, regardless of more existing inequalities which obviously are very difficult to repair. But at present there are no more important principles or fundamentals. And parallel to that it also must be aimed at a global office for the control and supervision of cartels, because existing national authorities are not able to prevent the tremendous global power concentrations which already became established and continue to grow on global level and develop still mightier – a scenario which would be impossible on country level due to existing national laws.

Although, different, and sophisticated ideologies are listed above, business will have to find own way before qualitatively new idea is developed. But, business can loose if these ideas will not be monitored and proper measures taken. Both corrective action, and local solutions are discussed further.

Applicable Conclusions for Rape Market

Previous general discussions were concluded using experiences from transition of Czech farmers from planned environment on free World price market, and later to regulated EU market. Firstly, EU rape market situation specified. Secondly, certain hope is exposed to forces of external environment. Thirdly, consequences occurring without any help will arrive are discussed.

EU Support Scheme for Rape

Rape seed receives just indirect support compared to cereals in EU. There are three kinds of support:

-Rape is allowed to be grown on set aside land, which is subsidised, but only to the agreed amount one million ton of soy cake equivalent.

-Further, rape is entitled to be subsidised by EUR 45 per ha on restricted number of hectares.

-Rape oil for bio-diesel had zero consumption tax till year ago. Tax levels differ now by country in EU.

No intervention fund neither export refunds are needed for rape, and oil because processing fro both food oil, and bio-diesel is done, and partly supported in national based factories, which are often privatised, and sold to multinational capital.

Consistency between Volatility of Markets, Support Schemes, and Taxation Measures

High price of mineral fuels have opened new market potential for bio-fuels, especially for bio-diesel in first half of 2006. EU farmers expected to reject set aside subsidies, and support rape seed production for bio-diesel in new member states in central, and Eastern Europe by subsidy EUR 45 per ha on limited number of hectares. But, price of mineral fuels have dropped in second half of 2006 on the level of 2005. Till that time Government of Germany agreed growth scheme for consumption tax on bio-diesel. Czech Republic approved zero exemption from consumption tax on bio-diesel. Massive export resulted from these decisions.

Above mentioned examples from recent history show large volatility of markets, which decisions of politicians made even more serious. Let's come back to the initial contradiction between legalistic, and free trade principals of World market regulation. Is it possible to co-ordinate national taxation policies? Is it possible to regulate price war between crude oil producers, and other energy producers? Does it make sense to tune trade barriers removals when such dramatic changes occur? It must be done otherwise all the hope in new land use for energy, and food production must be abandoned, and farmers will become unemployed.

EOA (European Oilseed Alliance) have discussed above mentioned taxation policy together with supply capability of EU farmers to investigate their competitiveness against imports. A potential to produce rape is evident as well as the will to import lacking vegetable oil for EU markets. Both Canola from Canada, and palm oil from Malaysia is produced in less regulated environment then is currently in EU. This is why they are imported to EU markets.

Green Policy for Farmers from Rich Countries who Lost High Property Value, and All who Have to Learn New Life Style on the Way

The question remains whether exports of oil from low income countries damage both their own farm employees, and EU farmers. The answer is that value of unemployment subsidy in is a cost of free trade. This cost should be compared with income improvement of farm worker in low income country who exported the product. Such balanced solutions should be incorporated into WTO schemes for trade harmonisation. Actually, states are responsible for compensation of tariffs decreasing. But, rich countries are afraid of isolated measures, which will not be applied, and favour competing states. The example of this is problem are refunds. EU reduced refunds, and waits for similar step from USA. WTO can do nothing with it now because it cannot handle voice of united low income states. Further measures less distorting free trade should be developed by rich countries now because impact on their producers is much more severe.

Brainstorming about above mentioned features of liberalization was organized by Group of Bruges. Small Romanian farmers expressed their will to stay at home, which will be complicated by EU entry. Then they join work force army marching World, probably. Problem of competitiveness of rape seed farmers is subject of meetings, and mission of organizations of rape seed producers, and processors. National Union of Oilseeds Growers and Processors proved that large scale production is a success factor for farmer. Recently, co-operation of producers and processors of oilseeds is recommended by European Oilseed Alliance. Czech Union of Oilseeds Growers and Processors, which already have established these product column relations, believes that the next step to be implemented is co-operation of rape seed producers, and processors with green parties.

Meeting of Czech Union of Oilseeds Growers and Processors have discussed different articles with special attention to direct combustion of oil in engines. It is expected that this new technology can strengthen local businesses. If this would happen income of sector stabilises, and will keep small farmers in their home rural area. Otherwise, just global farmers and companies in areas with monopoly best conditions will survive. That happened with farms in Czech Republic after collapse of planned system before EU accession.

Literature

Linhart, Z.: Prospects of Rape Oilseed Growing and Agriculture in EU at All. Union of Oilseeds Growers and Processors, Hluk 2005
Stauder, M., Popp, H., Greif, F.: CHANGING WTO RULES and the question of open versus regulated agricultural markets, Group of Bruges, Paris 2006.

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