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# Effect of processing conditions on the chemical composition and nutritive value of canola meal for broiler chickens and pigs

**Background:** Studies have shown that the nutritive value of canola meal (CM) can be enhanced or diminished by the processing conditions used in the canola processing plants. Excessive heating during pre-press solvent extraction may result in reduced digestibility of amino acids (AA), particularly lysine.

**Objective:** To evaluate the effect of processing conditions on the chemical composition and standardized ileal digestible AA contents of CM for broiler chickens and growing pigs.

**Methods:** Three surveys involving 11 Canadian crushing plants were conducted to determine the effect of processing and meal pelleting on the chemical composition of CM. As expected, some variations were observed in the contents of Lys (2.0-2.29 %DM), glucosinolates (2.0-10.1 µmol/gDM), and dietary fiber (33.3-41.9 %DM), the components known to be affected by heat treatment. Based on their levels, 6 representative samples were selected and used in an AA digestibility study involving 240 1-d old broiler chicks and 18 ileal cannulated barrows (BW=23.3 kg). Chicks were housed in cages (7 per cage) and were randomly assigned to 8 semipurified diets (including 2 additional diets containing pelleted CM from 2 plants) formulated to contain 22% CP. Semipurified diets for the pigs were also formulated to contain 18 % CP using the same set of CM samples. A casein-corn starch diet was included in the pig study to determine ileal endogenous AA losses. Pigs were housed individually in pens and were fed the 9 diets in a completely randomized design for 3 periods. All data were subjected to ANOVA using the GLM procedure of SAS.

**Results:** Standardised ileal digestibility values for Arg, Lys, Met, and Thr averaged 88.0, 79.9, 89.7, and 75.2%, respectively in the broiler study and 87.5, 78.8, 85.4 and 74.8 %, respectively in the pig study. There were variations ( $P<0.05$ ) between plants in the standardized ileal digestible content of all AA in both chickens and pigs. In the broiler study, the standardised ileal digestible contents of Arg, Lys, Met, and Thr averaged 2.24, 1.80, 0.55, and 1.09%, respectively, and ranged from 2.18 to 2.50% for Arg, 1.74 to 2.00% for Lys, 0.49 to 0.65% for Met, and 1.00 to 1.38 for Thr while in the pig study, they averaged 2.22, 1.78, 0.52 and 1.07 %, respectively and ranged from 2.00 to 2.44 for Arg, 1.61 to 1.96 for Lys, 0.45 to 0.63 for Met, and 0.94 to 1.34 for Thr. Pelleting reduced ( $P<0.05$ ) the standardized ileal digestible content of all AA in chickens and all AA except Pro in pigs with crushing plant x pelleting interaction for all AA.

**Conclusions:** The high dietary fiber and the corresponding low glucosinolates observed in some crushing plants could have been caused by CM overheating. There was relationship between the chemical composition of CM and their nutritive values for broiler chickens and pigs

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## Dual purpose canola: Increasing the value of winter canola by harvesting forage and seed

**Background:** Dryland agriculture in the Pacific Northwest (PNW) of the USA is dominated by small grain cereals. Low precipitation falls mainly in winter and early spring, as a result summer fallow is common. Yield of winter canola crops in the PNW are higher than other USA regions. Despite strong rotational benefits canola acreage in the region has not risen to meet demand from local crushers and much of this is because of high profitability in growing winter wheat.

**Objectives:** To examine potential of dual-purpose winter canola in a biennial system for forage and seed production. Feasibility is determined by considering forage quality and yield, along with the associated seed yield. Canola and canola mixtures are examined.

**Methods:** Three different are presented. (1) Two winter canola cultivars were sown at three planting densities over four planting dates (May through September) in four years. Vegetative biomass during the first year was harvested and ensiled to determine silage quality. Canola was allowed to overwinter and seed harvested the following summer. (2) 'Amanda' winter canola was planted in early May and forage harvested at weekly intervals from 4 weeks after planting through mid-August. The following summer seed yield was determined on all forage harvest treatments. (3) Winter canola was planted in early June inter-seeded with either spring wheat or spring pea. Forage mixtures were harvested when the wheat seed was at the milk stage. Thereafter, canola was allowed to overwinter and seed harvested the following summer.

**Results:** (1) Cultivars produced similar forage yield and quality and canola silage (Canolage®) quality was exceedingly high. Total dry matter forage yield was greatest for May plantings (5.2 t DM ha<sup>-1</sup>), while August seeded canola yielded 2.4 t DM ha<sup>-1</sup>. Planting dates had significant, but inconsistent effects on seed yield. Early-planted winter canola withstood multiple forage harvests without an impact on seed yield most years and economics indicate it as a feasible management practice. (2) Average dry matter forage yield was 4.0 Mt ha<sup>-1</sup> with highest forage production of 6.7 Mt ha<sup>-1</sup>. Under these dryland conditions, timing of forage harvest had no impact on subsequent seed yield, and average seed yield was 3,378 kg ha<sup>-1</sup>. Maximum combined income from forage and seed occurred when forage was harvested after 1,011 growing degree-days. (3) Forage yield was maximized with canola seeded at 9 kg ha<sup>-1</sup> with spring wheat at 22.4 kg ha<sup>-1</sup>, producing 9.0 Mt ha<sup>-1</sup> dry matter forage, with a relative feed value of 278 and a crude protein of 18.7%. Subsequent canola seed yield from this canola:wheat mixture was 3,483 kg ha<sup>-1</sup>.

**Conclusions:** Dual purpose early planted winter canola in the PNW has potential to increase grower profitability and hence increase canola acreage in the region. The PNW imports large quantities of forage and feeds to supplement a growing need for livestock feed, therefore, a local market exists for forage and seed meal.

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# Environmental effects on oil quality of high oleic-low linolenic (HOLL) and low linolenic (LLIN) spring canola

**Background:** Partially hydrogenated vegetable oils, which contain trans-fats, have adverse effects on human health. Traditional canola oil requires partial hydrogenation to avoid off-flavors when used for high temperature frying and to increase shelf life. Rancidity and off-flavors in oil are caused by high linolenic content, which has led to the development of high oleic - low linolenic acid (HOLL) and low linolenic acid (LLIN) canola cultivars. Availability of these cultivars in the Pacific Northwest would be of particular interest to the potato fry industry which requires large volumes of these oils. However, these new HOLL and LLIN cultivars must have stable quality over the environmental conditions throughout the Pacific Northwest.

**Methods:** Four LLIN lines and four HOLL lines were grown along with two standard canola cultivars ('Westar' and 'Profit') over 2 years in multiple location field trials throughout the Pacific Northwest. Two later plantings were included to simulate increased biotic and abiotic factors such as heat stress and insect infestation. Prior to flowering, racemes were covered with Delnet® pollination bags to avoid cross pollination. At harvest, seeds from the covered plant racemes were harvested by hand and used for fatty acid testing. Fatty acid profiles were determined using gas chromatography. The remainder of each plot was combine harvested, weighed to determine yield potential, and a seed sample analyzed for oil content.

**Results:** Interactions between cultivars and environments were often significant, though they were usually small compared to the main effects between cultivars. Cultivar x site and cultivar x year interactions were not significant for linoleic and linolenic acid, indicating genetic stability over a wide range of environments. Cultivar x site and cultivar x year interactions were observed for oleic acid indicating a potential environmental interaction. It is not known whether the LLIN and HOLL lines in this study have the documented FAD-2 or FAD-3 genes, which have shown environmental instability in some previous studies. However, it should be noted that the original LLIN and HOLL lines developed in breeding programs tended to show poor adaptability, either through gene drag from the mutagenesis techniques used in their development, or from the 'novel' fatty acids interfering with seedling growth. HOLL and LLIN breeding lines showed good adaptability for yield and oil quality. Therefore either negative genetic drag resulting from mutagenesis has been selected against or these lines have been selected such that they are no longer negatively impacted by the modification of fatty acids.

**Conclusions:** The results from this study show that recently developed LLIN and HOLL cultivars are adapted to a wide range of environments in the Pacific Northwest and maintain high quality oil suitable for non-hydrogenated fry oils while producing competitive seed yield and seed oil content.

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## Analysis and evaluation on quality characteristics of 500 rapeseed lines

**Background:** Erucic acid, glucosinolates and oil content are important quality indexes to the evaluation of rapeseed. Breeders have made great efforts to improve rapeseed quality. So far, lots of work about quality characteristics of crops was done by the data processing software. Collecting representative rapeseeds widely and analyzing the quality of them, then multiple correlation coefficient and cluster analysis were done by data processing software. It's significant in rapeseed quality improvement.

**Objective:** At present, germplasm resources of *Brassica napus L.* are very rich. In order to know whether branch height had any effect on rapeseed quality, seeds from different branch height of the plant were collected and analyzed separately. On the other hand, correlation and distribution among quality characteristics of 500 rapeseed lines were studied, which could provide guidance for cultivar breeding quickly.

**Methods:** When rapeseeds matured, seeds were collected respectively from different branches of 50 rapeseed cultivars. After that, quality characteristics were measured by NIRS. The software SPSS 19.0 was used to data statistic analysis. Non parametric tests were applied to significance analysis. Quality characteristics of 500 rapeseed lines were studied by using multiple correlation coefficient and cluster analysis.

**Results:** Branch height has significant impact on protein, glucosinolates and oil content, but indistinctly influence on erucic acid. The highest oil content and glucosinolates in the whole plant was discovered in the upper part of main inflorescence, while the highest protein content was in the lower part of that. On the other hand, there was high correlation among some quality characteristics. Oleic acid content was extremely significantly positively correlated with linoleic acid content and saturated fat acid content, and extremely significant negatively correlated with erucic acid content. Linoleic acid content was extremely significantly negatively correlated with erucic acid content, and extremely significantly positively correlated with saturated fat acid content. Erucic acid content was extremely significantly negatively correlated with saturated fat acid content. Oil content was extremely significantly negatively correlated with protein content. 500 tested materials were divided into 6 groups initially by cluster analysis. Materials of excellent quality characteristics were concentrated in group I and those of high quality characteristics were in group III, IV and V, which could provide theoretical guidance in parents selection.

**Conclusions:** Branch height had significant effects on protein, glucosinolates and oil content. Considering this, seeds from these branches in which quality contents were higher should be collected only, rather than ones of the whole plant without selection. Generally, linolenic acid and saturated fat acid differed little and the others differed greatly. It will provide a good base for new cultivars selection. Meanwhile, some correlations among quality characteristics were in contradiction with breeding goals, which could increase the difficulty in quality improvement.

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## Nutritional value of rapeseed oil: Alpha-linolenic acid level and a proper n-6/n-3 ratio

Rapeseed oil, a monounsaturated vegetable oil, presents a high nutritional value by its high level of Alpha-Linolenic Acid (ALA, 18:3n-3: 9%), the essential short chain precursor of n-3 family, which could be converted to Long-Chain-n-3 (LCn-3: EPA and DHA) by humans and animals. For an healthy human diet, this high level of ALA, associated to the moderate level of Linoleic Acid (LA, 18:2n-6: 20%) of this oil, induces a very low ratio n-6/n-3 (18:2n-6/18:3n-3 around 3 or less), which helps to promote the enrichment of ALA in tissues and its proper conversion to LCn-3. Based on this aspect, rapeseed is more efficient than soya oil which has a similar ALA content but more LA and a higher n-6/n-3 ratio (>10 vs 3 for rapeseed).

Populations of Western countries have a severe deficit in omega-3 intake, both in ALA (0.3% vs 0.8% of total caloric intake recommended) and LCn-3. In some cases, ALA is almost the only source of omega-3 for terrestrial animals including human. It has been shown that an adequate intake of this omega-3 precursor (ALA) in early life is able to cover the needs for LCn-3 and specifically for the brain DHA, via a proper liver conversion to LCn-3. The protective effect of ALA (rapeseed consumption) particularly during gestation and lactation of mothers, has been shown to induce a favorable effect on the brain DHA level of the offspring which is essential during early life and later, for an adequate development and protection against metabolic diseases in adulthood. Deficiency of Omega3 (precursor ALA, LCn-3: EPA; DHA) is associated to (i) Heart pathologies: thrombosis, sudden death, arrhythmia, CVD, stroke and, (ii) Brain pathologies: in the young (abnormal development, learning, behavior), in adult (depression, epilepsy) and aging populations (Alzheimer). The beneficial effect of LCn-3 has been largely reported, and despite much less studies, the beneficial impact of ALA is now well evidenced, in prevention (nutritional studies with chronic consumption) as well as an acute treatment for some pathologies.

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# ALA-rich rapeseed diet for dams and mice offspring are protecting against anxiety observed with low-ALA-palm diet

Omega-3 deficiencies during gestation/lactation could have dramatic impacts on cognition and behaviour. We previously showed in n-3-deficient-rats that ALA-rich-rape-seed-diet (8%) restored brain DHA levels to normal values and was much more efficient than palm-diet.

**Objectives:** Comparison of rapeseed or palm diets on mother and pups behaviour.

**Methods:** Two groups of dams (Swiss strain) were fed during 6weeks before and during gestation/lactation with:

- (i) Deficient-ALA-palm (0.4%) diet
- (ii) Protective-ALA-rich (8%) rapeseed diet.

Post-weaning pups received diets similar to their mothers until PND40 (Post-Natal-Day40).

## Results

There was no change in the behavior of pregnant dams, except in the palm-group which showed a reduced activity in a spatial memory test (Y-maze).

Pups belonging to the palm-group showed a reduced time at PND3 in the surface righting reflex test when compared to rapeseed group.

No significant differences were found between the treated groups in the geotaxis test (motor coordination and vestibular function, assessed at PND5,7,9,11), the suspension test (muscular strength, PND9&11) or in the Y-maze (assessing the short term memory post-weaning).

Three weeks after weaning, an increased anxiety was noticed in female mice of the palm-diet group when placed into an open-field (locomotor activity and anxiety).

This result was confirmed for the palm-diet group with an elevated-plus-maze test, while females born from ALA-rich-diet dams (rapeseed) showed a reduced anxiety compared to ALA-poor-palm-diet dams.

## Conclusions

Females born from dams fed with rapeseed oil (8% ALA), present an absence of post-weaning angiogenesis while those born from palm (0.4% ALA) presented an increased level of anxiety. The low n6/n3ratio (2.3) for rapeseed (while21for palm) could be part of its protective effect via a better brain DHA status.

# Investigation on cadmium contamination and migration from rapeseed to Oil

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**Background:** China is one of the countries with longest history of rapeseed production in the world. With the rapid development of modern industry, both natural and anthropogenic sources lead to an increase of Cadmium in soils and water. Cadmium in soils and water can be more readily taken up by plants and accumulate in the food chain (Yuan et al. 2013). Investigation of cadmium level and migration in rapeseed in China is a significant guidance to rapeseed oil safety, selection of varieties and breeding of low Cadmium accumulation variety of rape.

**Objectives:** The aim of this study was to investigate the Cadmium contamination and migration from seed to oil of rapeseed in the 11 main producing provinces in China and evaluate the contamination level of Cadmium in rapeseed.

**Methods:** A total of 596 representative rapeseed samples were collected from 11 main rape production provinces including Hunan, Hubei, Jiangsu and Jiangxi, where cadmium levels of soil and water are relatively high. And 30 sets of samples including rapeseed, rapeseed oil and rapeseed meal were collected from the large-scale integrated edible oil processing company in Hubei, Hunan, Guangdong, and Qinghai provinces. The cadmium contents of these samples were analyzed by atomic absorption spectrophotometry.

**Results:** Results indicated that Cadmium level in rapeseed ranged from 0.018 mg/kg to 0.142 mg/kg (P5~P95), the migration proportion of Cadmium from rapeseed to rapeseed oil was from 2% to 10%. Moreover, the migration rate negatively correlated with protein content in rapeseed with the correlation coefficient of 0.73. More importantly, the Cadmium contents of 99% samples of rapeseed were lower than 0.2mg/kg, which was significantly lower than Chinese standard maximum limit (0.5mg/kg).

**Conclusions:** In this study, we conducted investigation of the Cadmium level and migration in rapeseed in China. The results showed that the Cadmium content of rapeseed was far lower than Chinese standard maximum limit and rapeseed oils were safe and not influenced by the increase of Cadmium in soils and water.

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# Improvement of iturin A production by *Bacillus subtilis* with combined shaking and static culture mode using rapeseed meal as nitrogen source

**Background:** Iturin A is a new biopesticide with low toxicity, biodegradability and environmental friendliness. But its high production costs, low productivity and difficulty in fermentation process limit its commercial production. In our previous study, the effectiveness of direct bio-utilization of rapeseed meal as a nitrogen source for iturin A production by *Bacillus subtilis* was demonstrated by using glucose as carbon source (Jin et al. 2014). Biofilm fermentation is a newly developed promising technique in fermentation technology (Zohora et al. 2009), and the production of iturin A in static biofilm fermentation was reported two times higher compared to that in the submerged culture (Rahman et al. 2007).

**Objectives:** To further decrease the production cost of iturin A, the effect of different carbon sources and static biofilm fermentation on iturin A production was investigated when using low-cost rapeseed meal as a nitrogen source.

**Methods:** A combined culture mode of shaking culture first and followed by static biofilm fermentation was proposed based on the change characteristics of *Bacillus subtilis* growth and iturin A production during single static culture.

**Results:** The results indicated that wheat bran was the best carbon source for iturin A production, and the maximum iturin A concentration was 1.6-fold higher than that with glucose as a carbon source. Thick and stable biofilm was observed when adopting static culture, and the iturin A productivity was higher than that with traditional shaking culture during the later period of fermentation. Compared to single static culture, the proposed combined culture mode could further improve iturin A production, and the maximum iturin A concentration reached 1.10 g/L, close to the highest level produced with single shaking culture.

**Conclusions:** The highest iturin A concentration produced from combined culture could reach the maximum level with single shaking culture. Importantly, this new culture strategy was more easy to implement, which can not only decrease the production cost of iturin A but can also prevent the formation of a lot of foam in the later period of fermentation.

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# Nutritional value of rapeseed meal varies between oilseed rape varieties and processing conditions

**Background:** Rapeseed meal use in poultry has traditionally been limited because of concerns over anti-nutrients (Tripathi and Mishra, 2007). However, modern "double-zero" oilseed rape varieties have reduced glucosinolate levels, suggesting that rapeseed meal becomes more attractive for broiler diets.

**Objectives:** Nutritional value of rapeseed meal may be sensitive to oilseed rape variety and processing conditions (Newkirk et al. 2002). Therefore, variety and processing effects of oilseed rape on standardised ileal digestible amino acids (SID-AA) and apparent metabolizable energy (AME) content in rapeseed meals for broiler chickens were assessed.

**Methods:** Fifteen rapeseed varieties were obtained; eleven were hexane-extracted at CREOL producing rapeseed meal (RSM), three were cold-pressed producing rapeseed expeller (RSE), and one (var. Compass) was both hexane-extracted (RSM-Compass) and cold-pressed (RSE-Compass). SID AA was assessed using semi-synthetic diets with RSM and RSE at 500g/kg. A total of 192 14-day-old Ross-308 male broilers were fed test diets for 8 days (n=6 cages per diet; two birds per cage). AA digestibility was calculated by AA and inert marker (TiO<sub>2</sub>) quantification in diets and ileal digesta. SID AA coefficients were calculated by correcting apparent AA digestibility for basal ileal endogenous losses, and combined with diet AA to obtain diet SID AA content (g/kg dry matter, DM). AME was assessed through including RSE and RSM at 100g/kg in a maize-soybean meal reference diet, proportionally replacing all other energy-yielding ingredients. A total of 273 14-day old Ross-308 male broilers were fed the 17 resulting diets (n=7 cages per diet; three birds per cage), and excreta were collected on days 20 and 21 of age. Energy digestibility and AME content of the test diets were determined using the index method and for RSM and RSE samples through the difference method.

**Results:** SID coefficients across essential AA in RSM and RSE differed between varieties (p<0.05), averaging 0.84 (0.80-0.88) for RSM and 0.85 (0.83-0.87) for RSE. However, since protein levels varied between varieties and processing, larger differences emerged for total SID essential AA contents, i.e. 177 (159-198) g/kg DM for RSM and 120 (105-130) g/kg DM for RSE. RSE-Compass had greater SID coefficient than RSM-Compass for lysine (+6.2%), methionine (+4.6%), histidine (+3.2%), and arginine (+2.9%; p<0.05). AME content of RSM and RSE differed between varieties (p<0.05), averaging 13.1 (13.01-13.54) MJ/kg DM for RSM and 16.4 (15.90-17.21) MJ/kg DM for RSE. Energy digestibility and AME content were ~3% and 30% greater in RSE-Compass than in RSM-Compass (p<0.001).

**Conclusions:** Results suggest that oilseed rape variety and processing type can greatly influence nutritional value of resulting meals for broiler chickens. Ongoing work aims to correlate these findings with detailed biochemistry to predict nutritional value and inform breeding programs.

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# Comparison on the carbohydrate metabolic enzymes and their gene expression patterns in canola differing seed oil content

**Background:** Carbohydrates are thought of as an important regulator of canola seed oil content. Although it is well known that sucrose is a starting point before fatty acid biosynthesis, understanding the physiological regulation by carbohydrates governing seed oil accumulation is fragmented.

**Objectives:** to (1) compare the cleavage activity of sucrose and starch in canola high oil content line and low oil content line; (2) investigate the expression pattern of carbohydrate-related genes; (3) explore the possible roles of carbohydrate content and related enzymes on seed oil content.

**Methods:** In the present study, two canola recombinant lines differing seed oil content were used as experimental material. Activities of sucrose and starch catalytic enzymes, including neutral and alkaline invertase, sucrose synthase, and starch phosphorylase, and biosynthetic enzymes, including sucrose phosphate synthase, and AGPase, were compared in developing silique and seed of HOCL and LOCL, respectively. Sucrose and starch synthesis and catalysis genes were also compared at key stage of seed lipid deposition.

**Results:** The results showed that HOCL had significantly higher total soluble sugar concentration in the developing silique wall and seed during the seed lipid accumulation stage. Strikingly, all the enzymes showed very strong activities at 25 days after anthesis (DAA) in the silique wall of HOCL. At 25 DAA, enzyme activity was usually one-fold higher than at other stages. The result indicated that the high efficiency of cleavage of these carbohydrates in HOCL was beneficial for the rapid volume expansion and transportation of this compound to the seed for utilization. However, higher enzymatic activities were observed at the silique maturation stage in LOCL, thereby revealing the effect of these enzymes on cell wall thickening (i.e., cellulose accumulation). Similar activities of catalytic and biosynthetic enzymes suggested a homeostasis of carbohydrates in the silique wall. At seed deposition stage, all the enzymes exhibited significantly higher activities in HOCL than in LOCL, which was helpful for increased production of carbohydrates. The results of gene expression revealed that cell wall invertase was strongly expressed at late seed developmental stage in LOCL, and such expression could be closely associated with cellulose deposition. However, cytoplasmic invertase in HOCL exhibited higher levels of transcripts than in LOCL at all developmental stages. Only SUS3 showed significantly higher transcript amount in HOCL than in LOCL at all stages. The starch phosphorylase expression increased with seed development, but no significant difference was found in transcript levels between the two lines, except for SSIV at 50 DAA.

**Conclusions:** 1, extremely high activity of carbohydrate enzymes in HOCL was beneficial to produce more carbohydrates; 2, significant higher activity of carbohydrate catalytic enzymes in HOCL seed could help to produce more carbohydrate as oil synthesis substrate at seed lipid deposition stage; 3, high expression amounts of SUS3 and starch phosphorylase possibly played key roles in the higher enzyme activity in HOCL seed.

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## Control effect of root-knot nematode by using rapeseed cake in continuous cultivation at greenhouse

**Background:** Rapeseed cake could be utilized as animal feed and fertilizer for growing horticultural crops. Rapeseed cake could be a good source for increasing farm incomes. More efficient utilization could add more value to the rapeseed production chain which in turn would raise the competitiveness of rapeseed cultivation and production. Rapeseed cake contains several glucosinolates. The glucosinolates compound is known to control nematode due to their toxicity (Ludwig-Muler et al. 1997).

**Objectives:** The objective of this study was to determine the effectiveness of rapeseed cake in controlling soil nematodes.

**Methods:** Tomato (cv. Rutgers) was used as a host plant in order to study the control effect of nematode by using rapeseed cake. The tomato plants were grown for 20 days in pots filled with mixed soil (1 kg) of clay loam soil (500g) and sandy soil (500g). And then the soil was supplemented with 50g of rapeseed cake from Jeju local rape varieties and 'Sunmang' variety, respectively. Roots and soil around roots were inoculated with egg sacs of nematodes, and then the tomato plants were grown for 60 days. The density of nematodes was investigated by separating nematodes from cultivated soils and the roots.

**Results:** Two different rapeseed cakes (Jeju local rape varieties and 'Sunmang' variety) were mixed with the soil to control nematodes environmentally. When soil physical properties in the rapeseed cake-mixed soils were analyzed, OM (organic matter), P205, Ca, Mg, and CEC (Cation Exchange Capacity) value increased. Especially, the level of OM was 3-fold higher than control soil. Glucosinolate content of rapeseed cake was higher in Jeju local rape varieties than 'Sunmang' variety. The major components of glucosinolates were consisted of progoitrin, gluconapin, glucobrassicin napin, and sinigrin. These components were likely to be involved in reducing nematode density.

**Conclusions:** In this experiment rapeseed cake reduced the density of getting infested with nematode in the soil because the glucosinolates compound in rapeseed cake has toxicity to kill nematodes. The Glucosinolates are secondary metabolites including  $\beta$ -D-thioglucoside and sulphonated oxime and it is converted into isothiocyanates (ITCs) thiocyanate and indole etc (Mithen et al. 1987).

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POSTERS THEME C

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## Does *Camelina sativa* contamination of double low rapeseed expellers cause milk fat depression in dairy cows?

**Background:** Expellers and press cakes of double low *Brassica napus L* rapeseed (RPCs) are normally recognized as valuable parts of the concentrate feed to dairy cows. However, during spring 2013 milk fat depression was observed among some high yielding Danish dairy herds fed commercial RPC's containing 13-14% fat as part of their feed. The observed milk fat depression varied, showing depression up to 0.5 % units.

**Objectives:** The objectives of the present study were identification and evaluation of possible correlations between bioactive constituents in the applied feed and the dairy cows' milk fat depression.

**Methods:** Nine RPC containing samples from feed fed to nine different milk fat depressed herds were collected, analyzed and the analytical data were compared with corresponding data from six reference groups of herds without milk fat depression. The RPC samples were analyzed for protein, fat, fatty acids (FA's), phenolics and glucosinolate content.

**Results and discussion:** The RPC containing samples did not differ significantly in protein content and total fat content. RPC's in feed fed to milk fat depressed herds differed, however, from that of the reference feed with respect to FA composition. They were thus higher in C18:3n-3 (12.1% vs 8.9%), C20:1n-9 (2.7% vs 1.1 %), C22:1n-9 (0.7% vs 0.1) and lower in C18:1n-9 (51.5% vs 57.4%).

With respect to phenolics and glucosinolates, some striking differences were seen in the profiles of individual compounds; the flavonoids (especially quercetin glycosides) and ω-(R)-methylsulfinylglucosinolates.

RPC's from milk fat depressed herds showed a characteristic content of 4 different ω-(R)-methylsulfinylglucosinolates (n=8, 9, 10 and 11) varying in total concentrations from 0.7-3.3 μmol/g RPC's. Seeds of high quality double low *Brassica napus L* only contain ω-(R)-Methylsulfinylglucosinolates with n=4 (glucoraphanin) and n=5 (glucoalysin) and in low concentrations (0.1-0.2 μmol/g seed). Seeds of *Camelina sativa (L.)* Crantz, however, accumulate appreciable concentrations of the 4 homologues with long side chains; n=8, n=9, n=10 and n=11 (Das et al., 2014, Andersson et al., 2008) with total concentrations from 10-30 μmol/g seed depending on variety.

The observed milk fat depression was very similar to the milk fat depression caused by conjugated linoleic acid (CLA), but although the content of unsaturated FA was higher in RPC samples from milk fat depressed herds it was not considered to be high enough in the total diet to cause milk fat depression. However, based on the quantitative analysis of FA, phenolics and glucosinolates in double low rapeseed and *Camelina sativa* seeds, it seems more likely that the observed milk fat depressions are caused by RPC's contaminated with around 15-20% of *Camelina sativa* seeds.

**Conclusions:** The cause of the milk fat depression is not completely elucidated, but it is likely that it is caused by a contamination with *Camelina sativa*, but whether it is caused by the glucosinolates or other compounds in *Camelina sativa* seeds still needs to be resolved.

**Implications:** This study shows the importance of natural product fingerprints as an important means to detect adulteration of feed impairing significantly on animal metabolism and thereby on their production yields.

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# Harnessing potential of mustard glucosinolate as biopesticides

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**Background:** *Brassica* (rapeseed mustard) is the key edible oilseed crop in India after groundnut. Oil-cake contain a high-quality protein that forms an important cattle-feed and manure. However, it also contains relatively high amounts of anti-nutritive fibre compounds, phenolic acids, phytate and glucosinolates. Glucosinolates, compounds that occur in the cake, represent a viable source of allelochemic control for various soil-borne plant pests. As such, glucosinolates are commonly considered to be ultimately responsible for pest suppression (Fenwick et al, 1983). The detrimental effect of cruciferous tissues on other microorganisms has also been attributed mainly to volatile degradation products of glucosinolates released from these plants. With this background present study was undertaken to explore the potential of glucosinolate as bio pesticide.

**Objectives:** De-oiled rapeseed meal produced in mustard oil extraction is used as cattle and poultry feed in India but due to high content of glucosinolate it has limited preference in international market. Preliminary studies show that mustard cake has anti-pesticidal potential due to its high glucosinolate content so objective of the present work is to development of the process for isolation of glucosinolate en-rich fractions and assess its potential as biopesticide.

**Methods:** Seed, Seed cake and oil samples were analyzed for glucosinolate content as per the method of Kaushik and Agnihotri (1999). A sequential extraction method was developed for the extraction of glucosinolate rich fraction. Samples were bioassayed for the anti-feedency and growth inhibition activity by pair choice and diet mixing bioassay respectively against *Spodoptra litura*.

**Results:** In order to determine the best source for glucosinolate extraction Indian and European *Brassica* species were analyzed for their glucosinolate content. Indian species of brassica (*B. juncea*, *B. nigra*, *B. campestris*) and European mustard (*B. juncea*) has very high amount of total glucosinolate. Indian mustard contains both allyl and butenyl while European mustard contains only allyl glucosinolate. Rai seed (*B. nigra*) is having high glucosinolate content as compared to mustard (*B. juncea*) and toria (*B. campestris*) seed. Extract prepared to isolate glucosinolate rich fractions was also analyzed and it was found that glucosinolate content of brown toria (*B. campestris*) extract is very high. Methanol extract of the brassica seed cake showed highest feeding inhibition whereas very high growth inhibition was obtained with brown toria formulation.

**Conclusion:** Glucosinolates exhibits very good antifeedency and growth inhibition against *Spodoptera litura* insect and the glucosinolate rich extracts can be explored for the up-scaling prospect for use as biopesticide.

**Acknowledgements:** Present studies was undertaken as part of APROPOS project, which has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreements n° 289170.

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# The influence of variety and environment on the biochemical analysis of oilseed rape meal

**Background:** Despite major breeding advances, the use of oilseed rape meal (RSM) in pig and poultry rations remains limited by traditional concerns over their anti-nutrients (Tripath and Mishra, 2007).

**Objectives:** Biochemical analysis of a set of modern oilseed rape varieties was performed as part of a larger study to explore the potential for increasing confidence in using oilseed rape meal in monogastric diets. Using common origin material, from multiple sites, we have collected new data on variability of RSM biochemical composition, and the influences on this of genetics and environment.

**Methods:** Twenty-two varieties, from each of five locations from the 2012 UK National List variety trials program, were selected to provide maximum diversity using known data on oil content, whole-seed glucosinolate content, breeding type (hybrid/open pollinated) and origin (breeding program). This provided common origin material from a range of environments. Seed samples were milled and de-fatted by cold-hexane extraction to prepare meal samples. Using standard laboratory methodologies, samples were analysed for crude protein (CP) and amino acid composition, total glucosinolate content and composition, tannins, phytic acid and sinapine.

**Results:** Average CP content (22 varieties x 5 locations) was 34.5g/100g, with a maximum range (varieties x sites) of from 28.9 to 37.8g/100g, site and varieties effects contributing equally to this. Amino acid composition was very consistent between varieties. Glucosinolate content averaged 20.4µmol/g with a large overall range (10.8-52.5µmol/g) the variation coming principally from variety effects. This range was exaggerated by the inclusion of a new variety type with an altered oil profile and relatively high glucosinolate content. Excluding this variety reduced the overall mean value to 19.4µmol/g and the upper range limit to 36.1µmol/g. The individual glucosinolate components showed relatively little variation, the most coming from progoitrin and 4OHglucobrassicin. Tannins averaged at 1.59 mg/g catechin equivalents but also exhibited considerable variation (0.28-3.21 mg/g), largely from site effects. Phytic acid averaged 2.83 g/100g and varied less (1.32-3.78 g/100g), with the main variation again coming from sites. Sinapine averaged at 7.58 mg/g (5.10-9.30 mg/g), with similar variation observed for sites and varieties.

**Conclusions:** Levels of CP conformed closely to the 33.9g/100g published values for rapeseed meal in feed tables (Premier Nutrition, 2008) and show comparatively little variation. The principal cause of variability in glucosinolate content is genetically controlled (varieties) and levels in RSM batches for feed can continue to be improved by progressively tightening the standards set for commercial variety releases. Current differences due to variety or growing environment will be largely nullified by mixing grain loads at the crushing plants. Tannin, phytic acid and sinapine values were generally low with little potential for variety improvement in the current generation of cultivars.

## Acknowledgments:

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# Indian mustard (*Brassica juncea*) oil to combat deadly diseases

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**Background:** Cancer and cardiovascular disorders remain the main cause of mortality, morbidity and health care burden worldwide. Heart disease is a leading cause of death in India. These two diseases are directly linked to diet and related factors, therefore, it is imperative to make dietary changes that could reduce the incidence of these two diseases. Indian mustard (*Brassica juncea*) is an important source of edible oil in India. Consumption of mustard oil seems to heart friendly as it has the lowest content of saturated fatty acids (SFA) among the vegetable oils. Also among the saturated fatty acids, stearic acid is considered as neutral towards heart related problems.

**Objectives:** In addition to the premium quality edible oil, seed meal obtained after extraction of oil is a rich source of many health promoting bioactive compounds including glucosinolates, phenolics, flavonoids, carotenoids, etc. Incorporating these phytochemicals in a daily food is a safe, effective and inexpensive way to guard against two of today's most common health problems cancer and cardiovascular disorders. However, efficacy of these phytochemical is also often limited by the potential to reach the site of action as only a small fraction of intake reaches the target site. Physical instability, low solubility in aqueous medium, lower absorption rate and bioavailability are some of the factors responsible for slow pharmacological actions.

**Methods:** Under quality prospection programme, more than two thousand samples of germplasms, advance breeding and elite lines of Indian mustard grown in different agro climatic regions of India were screened for oil and seed meal quality parameters during 2000-2010.

**Results:** Total SFA content was less than four percent. Besides that, ratio of essential fatty acids content namely linoleic acid ( $\omega$ -6) and linolenic acid ( $\omega$ -3) was found to be very close to 1.25, the value recommended by WHO. Based on the fatty acid profiles, it was calculated that one tea spoon (about 5 g) of unrefined mustard oil could supplement about 900 and 750 mg of linoleic and linolenic acid respectively to daily diet.

**Conclusion:** Nanotechnology provides a platform to overcome these challenges and can be utilized for novel drug delivery systems with improved bioavailability as well as site specific distribution. Furthermore, value added products obtained from seed meal using low temperature drying techniques namely spray or freezing drying could be utilised for production chocolates bars, capsules, semi solid colloidal gel to enhance the acceptability of the products among the targeted groups. Besides, improving the palatability, these products will also have consistency in terms of the concentration of the targeted compounds for their site specific actions.

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# Novel feed forms from canola for dairy cattle: Glycerol and high-oil canola meal

**Background:** Canola meal is a good protein supplement for dairy cattle. It is desirable for the canola industry to generate new high value feed products from canola and identify new markets and applications. The high level of milk production in present dairy cows creates high metabolic demand for energy (starch, fat, fiber) and protein with specific kinetic properties in digestion. Dairy producers have a need for specialty feed supplements that can be used to manage the cow's unique metabolic needs during specific periods such as after calving and during lactation. Glycerol is a new feed by-product from the biodiesel industry and is a unique glucogenic, high energy source for dairy cattle (Carvalho et al., 2011). High oil canola meal (HOCM) typically is a cold press extrusion by-product from biodiesel production (Hristov et al., 2011). The fat in HOCM is a unique energy source for dairy cattle, complementary to starch. The HOCM increases polyunsaturated fatty acid content in milk fat, including Conjugated Linoleic Acid (CLA) which has nutraceutical properties.

**Objectives:** To determine the suitability of glycerol and HOCM, alone or in combination, as feed supplements for dairy cattle; to develop recommendations on optimal safe inclusion rates, and; to identify optimal feeding management protocols using these supplements to best support the metabolic need of high producing dairy cows.

**Methods:** HOCM with 10-14 % residual canola oil was from Milligan Biofuels and glycerol from Cargill Animal Nutrition. We performed four research trials at the University of Saskatchewan Dairy Research Facility. Two were in lactating cows between 2 and 7 months in lactation, where in one trial we performed a dose-response study with glycerol, and in the other studied combinations of glycerol with HOCM and distillers dried grains and solubles (DDGS), another by-product from the biofuel industry. Experimental design was a double 4x4 Latin square with periods of one month. The next two trials were performed in transition cows from -2 weeks to +6 weeks from parturition, when the cow experiences the highest metabolic stress and highest nutrient demand. Experimental design was a randomized block. In the third study we used 24 cows with three diet treatments (control standard Total Mixed Ration (TMR), TMR with glycerol, and TMR with glycerol and HOCM) and in the fourth trial 32 cows with two diet treatments (control TMR, and TMR with glycerol, HOCM and DDGS). In all trials we measured lactation performance and changes in blood metabolite composition and hormone profiles.

**Results:** Glycerol is a useful energy feed for lactating and transition dairy cows and acceptable up to 10% of the dry matter intake, the highest level tested, using a TMR typical for SK conditions. Glycerol feeding value is equivalent to that of corn on an energy basis. Glycerol improved lactation performance and yield of milk protein. We attribute this to the rapid availability of the energy from glycerol (high solubility) for rumen fermentation, supporting increased microbial growth and protein synthesis and improving protein supply for milk protein synthesis. Glycerol and particularly glycerol with HOCM improved energy balance and lactation performance in transition cows

**Conclusions:** The canola biofuel by-product feed supplements increased milk production performance and feed efficiency of cows. Particularly, the supplementation of the TMR with a combination of glycerol and HOCM appeared most effective. The biofuel by-products have unique feeding attributes which can be used to provide increased nutrient supplementation of cows during periods with high levels of milk production and nutrient needs, such as transition dairy cows and those needing to improve body condition.

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## Rapeseed or fish oil protects muscle mass during energy restriction in the rat

**Background and Objectives:** In obese subjects, the loss of fat mass during energy restriction is often accompanied by a loss of fat-free mass (mostly muscle), especially in the absence of physical activity. In other contexts leading to a muscle loss (immobilization, inflammation, cancer), several studies showed that n-3 polyunsaturated fatty acids (PUFA) modulate protein homeostasis, especially via effects on insulin sensitivity. The n-3 PUFA intake during weight loss could thus help protecting muscle mass. This hypothesis has been tested in rats with two sources of n-3 PUFA i.e. the vegetable precursor, linolenic acid (18: 3 n-3) and its long-chain derivatives (LC, 20: 5 n-3 and 22: 6 n-3).

**Methods:** Male adult Wistar rats (n=48) were fed for 4 weeks with a high-fat induction diet, then 3\*12 rats were energy restricted during 8 weeks (50% of ad libitum intake) while the 12 remaining rats were fed ad libitum. During these two phases, the dietary lipids contained oleic sunflower oil (71% 18:1 n-9, ad libitum ADLIB and restricted OLE control groups), rapeseed oil (10% 18:3 n-3, RAPE group) or fish oil (10% LC n-3 PUFA, FISH group). At the end of the restriction phase, rats were anesthetized prior to intravenous insulin injection, sampling of the Gastrocnemius muscle, and euthanasia. Post-mortem analyses were as follows: body composition, expression of muscle genes involved in proteolysis by qPCR, and activation by phosphorylation of muscle proteins involved in insulin signalling by western blotting.

**Results:** During the induction phase, the weight gain was similar in all groups. During the restriction phase, ADLIB group continued to gain weight while all energy- restricted rats lost weight (-20%) and fat mass (-50%). However, when compared to the ADLIB group, leg muscles significantly lost weight in the OLE group (-4 to -6%), but not in the RAPE and FISH groups. As concerns proteolysis key-enzymes, transcript levels involved in the ubiquitin/proteasome system were significantly decreased in the FISH group (-30% for MAFbx and -20% for MurF1), intermediary in the RAPE group and unchanged in the OLE group, when compared to the ADLIB group. The type of dietary fatty acids had no effect on calcium-dependent (calpain 2) and lysosomal (cathepsin D) systems. In response to insulin, phosphorylation levels of AKT and IRS1 (insulin receptor), known to stimulate protein synthesis, were significantly increased (+ 70%) in the FISH group, when compared to the ADLIB group. A similar result was observed for the transcript level of IRS1 (+ 50%), which promotes the transduction of insulin signal. The RAPE group exhibited the same activation pattern as the FISH one, with the exception of IRS1 phosphorylation level. No change was observed for OLE group.

**Conclusion:** Dietary n-3 PUFAs prevent the loss of muscle mass associated with energy restriction. This beneficial effect is associated with an improved activation of the insulin-signalling pathway. Most importantly, the vegetable 18: 3 n-3, supplied by rapeseed oil, has the same overall efficiency as n-3 LC-PUFA from fish oil.

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# Rapid determination of phenolic compounds in rapeseed oil using magnetic multi-walled carbon nanotubes as adsorbents followed by liquid chromatography-tandem mass spectrometry

**Background:** Phenolic compounds which are widely existed in edible oils, have drawn considerable interest for their antioxidant and health effects. Traditionally, the isolation and enrichment of phenolic compounds from the triacylglycerol matrix requires complicated sample treatment procedures (Bajoub et al. 2015), such as liquid-liquid extraction, dispersive solid-phase extraction (DSPE) or by solid phase extraction (SPE). Therefore, it is necessary to analyze minor constituents by a simple and rapid preparation method with high extraction efficiency and avoiding tedious steps (Zhao et al. 2012).

**Objectives:** The aim of this study was to develop a rapid and robust method for determining phenolic compounds in rapeseed oils using magnetic solid-phase extraction adsorbents (MWCNT-MNPs) coupled with high performance liquid chromatography tandem mass spectrometry (LC-MS/MS).

**Methods:** MWCNT-MNPs were simply obtained by wrapping amine-functionalized Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles into multi-walled carbon nanotubes. The major parameters affecting extraction efficiency were investigated, including the type and volume of desorption solvents, extraction and desorption time, washing solution, and adsorbent amounts.

**Results:** The extraction procedure can be achieved by a 6 min simple vortex, and the cleanup needs only 30 s vortex without tedious concentration and derivative steps. The limits of detection (LOD) of phenolic compounds, based on a signal-to-noise ratio (S/N) of 3, were in the range of 0.05-0.40 µg kg<sup>-1</sup>. The recoveries of phenolic compounds in oil samples were in the range of 85.0-110.0% with RSD less than 12%.

**Conclusions:** In the validation of MWCNT-MNPs-LC-MS/MS method in real oils, the results indicated that phenolic compounds, such as gallic acid, catechin, caffeic acid, sinapic acid and ferulic acid were widely existed in rapeseed oils, and chlorogenic acid, cinnamic acid were not detected in rapeseed oils. On average, virgin rapeseed oils have higher level of gallic acid, catechin, caffeic acid, sinapic acid and ferulic acid than refine rapeseed oils. Phenolic compounds in oils were linked to preliminary heat treatment of oilseeds and the refining process of oil. The proposed method is reliable, robust and potential for the analysis of phenolic compounds in rapeseed oils.

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# Phytosterol analysis method and its application in adulteration detection of rapeseed oils

**Background:** Phytosterols make up the largest proportion of the non-saponifiable fraction. Due to the LDL cholesterol lowering effects and high content in edible oils, phytosterols were regarded as important nutrient components and quality parameters of edible oils (Normén et al. 2007). However, the past reports mainly focused on the several abundant phytosterols such as  $\beta$ -sitosterol, stigmasterol, campesterol, and brassicasterol. Therefore, it is necessary to develop a more comprehensive analytical method for the phytosterol profiles (Xu et al. 2014).

**Objectives:** The more comprehensive analytical method for phytosterol profiles should be developed for the subsequent quality inspection, nutrient and functional evaluation of edible oils. The aim of this study was to develop a robust analytical method for phytosterol profiles and use the method for adulteration detection of rapeseed oils.

**Methods:** Free phytosterol profiles of rapeseed oils were established by SPE–multidimensional gas chromatography coupled to time-of-flight mass spectrometry (GC–GC–TOF/MS) and employed to classify the rapeseed oils and other three edible oils with the help of unsupervised (Principal Component Analysis and Hierarchical Clustering Analysis, PCA and HCA) and supervised (Random Forests, RF) multivariate statistical methods.

**Results:** As results, 13 phytosterols were completely separated and quantitatively analyzed by GC–GC–TOF/MS. The RF model results indicated that the free phytosterol profiles of the four edible oils (rapeseed oil, soybean oil, peanut oil, and sunflower seed oil) could completely and correctly classify the oils into four groups, and therefore could be taken as effective markers for identification of rapeseed oils. Moreover, a simulated data test indicated that free phytosterol profiles could detect rapeseed oil adulterated with 10% of other oils.

**Conclusions:** In this study, a simple and rapid SPE method was developed for separating free sterols from edible oils, and their silylation derivatives have been analyzed by GC–GC–TOF/MS, leading to a good separation resolution. Moreover, combining with chemometric methods, this method was used to detect the adulteration of rapeseed oil. The validation results indicate this method could effectively detect the fraud rapeseed oil adulterated with more than 10% of other edible oils.

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# Studies on volatile flavor components of cold-pressed rapeseed oils

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**Background:** The flavor is one of the most important sensory characteristics of edible oils, which mainly originates from volatile flavor components (VFC). Generally, different oils have its own characteristic flavor, contributing to identify oil species and analyze quality changes. Up to now, domestic and international researchers found that the main source of VFC came from oxidation products of unsaturated fatty acids in edible oils, followed by maillard reaction products between sugars and amino compounds in the process of heat treatment (Shahidi et al, 2005). Some domestic researchers analyzed the influence on VFC of rapeseed oils under different processing technologies, which demonstrated that the main VFC of rapeseed oils were glucosinolate degradation products (GSDP), oxidized volatile substances and heterocyclic carbenes. However, there were few reports on the VFC of cold-pressed rapeseed oils (CROs), and the relationship between GSDP and sensory properties of CROs.

**Objectives:** The VFC was the most important indicator of CROs, good-flavored CROs was directly related to the acceptable level of market. The VFC of CROs with different amounts of GSDP was measured and compared it to that of different treatments, which provided theoretical basis for developing CROs products with good tastes.

**Methods:** The VFC of three kinds of CROs with different GSDP contents was analyzed by combination technique of head-space solid-phase micro-extraction, gas chromatograph-mass and olfactory detection port, and the CROs were cold-pressed low-erucic rapeseed oil (LERO), cold-pressed medium-erucic rapeseed oil (MERO) and cold-pressed high-erucic rapeseed oil (HERO). In addition, we compared VFC of various kinds of rapeseed oils, including LERO, hotpressed low-erucic rapeseed oil (HLERO), solvent-extracting low-erucic rapeseed oil (SLERO), refined rapeseed oil (RRO) and microwave-pretreatment LERO (M-LERO). The sensory evaluation of different oils was carried out by several assessors with professional backgrounds of oils.

**Results:** The VFC of CROs was composed of aldehydes, alcohols, hydrocarbons, furans and more than 60% GSDP. By comparing rapeseed oils processed with several techniques, it was demonstrated that LERO, HERO, HLERO, SLERO and M-LCROs all possessed higher contents of GSDP except for RRO, however, the types and contents of VFC of each oil were different. In addition, oxidizing volatility products of five oils were mainly aldehyde. The flavors of CROs were mainly woody, astringent, bitter, and the most intense seed-like. Low-temperature refining had no obvious influence on CROs flavor, and M-LCROs presented pleasant nutty and roasted flavor.

**Conclusions:** Each processing technology showed great effect on the VFC of rapeseed oils, producing different contents of components. However, GSDP possessed an important part in the VFC of rapeseed oils except for RRO and aldehyde was the common oxidizing volatility products of the above studied five oils. This work contributed to the development of CROs products with desired flavors.

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## Production of value added co-products in the seeds of the industrial oilseed crop *Camelina sativa*

**Background:** With growing concerns of diminishing fossil fuel feedstock availability and increased global climate change, oilseeds are emerging as a sustainable platform for producing fuels, chemicals and materials. Production of industrial commodities through agriculture provides an opportunity to produce these materials in large quantities at a low cost. *Camelina sativa*, belonging to the *Brassicaceae* family, is being targeted as an industrial crop due to its high oil content that is suitable for production of renewable industrial lipids and oleochemicals as well as aviation and other liquid fuels. However, there is currently insufficient value within *Camelina* to compete with other crops for acres. Options for increasing the value of the crop include engineering a value added co-product or significantly increasing its yield and oil content. Metabolix Oilseeds and its parent company, Metabolix, are currently pursuing both strategies to make *Camelina* an economically viable crop.

**Objectives:** This study focussed on seed-based production of the polymer polyhydroxybutyrate (PHB) in plastids of *Camelina* seeds as a co-product to increase the value of the seed. PHB is one member of the broad polyhydroxyalkanoate (PHA) family of renewable biodegradable materials with properties that make them suitable substitutes for many applications currently served by petroleum derived plastics. PHB is an ideal co-product for an industrial oilseed since it has multiple market applications. Besides its use as a low cost polymeric material, it has also been targeted for use as an enhanced feed supplement and as a feedstock for the production of renewable chemicals (Snell and Peoples 2013; Snell et al. 2015).

**Methods:** Multiple genetic constructs with different seed-specific promoters encoding plastid targeted PHB biosynthetic enzymes were constructed and transformed into different accessions of *Camelina sativa*. The best genetic constructs for PHB production were identified and homozygous PHB *Camelina* lines were produced. PHB levels were quantified and the effect of PHB production on carbon partitioning in seeds was determined.

**Results:** PHB levels of up to 15% of the mature seed weight were obtained in bulk *Camelina* seeds (Malik et al. 2015), the highest level of PHB produced in a seed to date. Transmission electron microscopy showed the presence of distinct granules of PHB in seed plastids. High level production of PHB had varying effects on germination, emergence and survival of seedlings.

**Conclusions:** The high level of polymer produced in seeds of *Camelina* is an important step forward for commercializing an oilseed-based platform for PHB production. Additional work is on-going to increase seedling emergence and vigor in high producing lines.

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# Variability of fatty acids and tocopherols in NS rapeseed collection

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**Background:** Because of its high-quality nutritional composition, rapeseed is a common source of edible oil. Recently, the focus in rapeseed breeding has turned to improving and altering the content and composition of salutary oil constituents, such as oleic acid and linolenic acid contents and tocopherols (Fritsche et al. 2012). One of the main goals in the rapeseed breeding program at the Institute of Field and Vegetable Crops, Novi Sad (Serbia) is to create genotypes with a specific level and combination of different fatty acids and tocopherols. Classification and characterization of rapeseed germplasm and the selection of superior genotypes for utilization in the field production or as parents in future hybridization program can be effectively performed by using multivariate analysis (Jankulovska et al. 2014).

**Objectives:** The main objectives of the study were to exploit the variability of fatty acids and tocopherols content within the NS rapeseed collection, to classify the genotypes based on their oil quality and to identify genotypes with desired fatty acids and tocopherols content.

**Methods:** Total of 49 genotypes were analysed for alpha and gamma tocopherols and oleic, linoleic, linolenic, stearic, palmitic, arachidic, behenic, eicosenoic, lignoceric and erucic acid content. Classification of rapeseed germplasm was performed using multivariate statistical methods. Principal component analysis (Revelle 2014), cluster analysis (Suzuki and Shmodaira 2013) and two-way cluster analysis (Day 2012) were applied.

**Results:** Principal Component Analysis revealed 5 PC components with Eigen value >1, which explained 78.70% of the total variability. Cluster analysis and two-way cluster analysis helped identify genotypes with similar fatty acid and tocopherol composition. Two main groups could be identified on the dendrogram, the first having two genotypes and the second comprising 44 genotypes. Three genotypes did not belong to any group.

**Conclusions:** The applied techniques can be helpful for identification, selection and optimized exploitation of rapeseed genotypes with desirable oil quality.

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# Advanced calibration method for glucosinolate analysis in rapeseed seeds using a Near Infrared Reflectance spectrometer

**Background:** The fast and cheap and nondestructive analytical methods of large quantity of samples are in big need for breeding and research purposes. Today there are two instrumental methods available – NMR for fat and moisture estimation in seeds or NIR- able to measure all important chemical components. The basis of obtaining reliable results by NIR is good, robust calibration model. The “Local” method was introduced by John Schenk (Schenk and Westerhaus 1997) and was developed to evaluate large databases (many thousands of samples) of spectra and reference values using the single sample prediction concept (method of analyzing an unknown material patent US 5798526 A).

**Objectives:** For calibration purposes couple mathematical methods (multiple regression, principal component, neural network) was developed. In this study one less popular method called “Local” was investigated and compared to classical PLS method to check if this method can generate more precise results.

**Methods:** Spectrometric data were obtained by NIRS 6500 spectrometer with ISISCAN 4.6 software. All calculations were made by using the Winlsi (Foss) package. 2400 samples were used as calibration set and 600 samples as validation set. The samples were collected over 7 years from western Poland locations. Corresponded reference analysis of glucosinolate content by gas chromatography of desulfoglucosinolates (Raney and MacGregor 1990 Michalski et al 1995) was accomplished.

**Results:** The sample set of 2400 samples was used to generate PLS calibration models. The PLS calibration generates whole spectrum equation estimating the value of calculated components. It need linear data and work properly with samples similar to used in calibration. The same set was used to generate Local database. Obtained validation results for both methods were compared and the Local error was slightly less than Global error for all glucosinolates (Gluconapin 1,1-1,2, glucobrassicinapin 0,4- 0,5, Progoitrin 2,3-2,4, naploeiferin 0,5-0,6 Glucobrassicin0,17-0,2, 40H-glucobrassicin1,0-1,2 and total 3,6 -3,7. All result in uM/g seed)

**Conclusions:** The comparison of prediction errors (SEP) shows that local is giving slightly better results. To make Local method work it is necessary to collect very large amount of representative samples what force multiyear collection process and high reference analysis cost. If the obtained database is truly representative it can be used to any material covered by samples it contains. It is possible to generate database using commercial software- WINISI, when Neural network calibrations need cooperation with machine vendor, as there no subsequent software is available. It was observed that database with majority of samples in the range of 5-30 uM/g glucosinolates generate calibration equations that poorly estimate the high glucosinolate samples when PCA global equations worked properly.

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# Evolution of high oleic low linolenic (HOLL) winter oilseed rape (WOSR) production in Switzerland: How agricultural innovations can open markets and increase production options

**Background:** HOLL winter oilseed rape (WOSR) produces a vegetable oil suitable for frying without partial hydrogenation, thanks to its specific fatty acid profile.

**Objectives:** Analyze how the production of HOLL WOSR evolved in Switzerland since its introduction in 2004, and the possible future trends.

**Methods:** Data of WOSR production were compiled and analyzed in terms of HOLL surface, yield, and oil quality. Surveys about crop management and quality were organized.

**Results:** HOLL WOSR was first grown in Switzerland in 2004. The surface allocated to HOLL constantly increased, to reach 8'000 ha for the 2015 harvest. This represents 32% of today's total WOSR surface. This development took place without a reduction of conventional WOSR production, showing that a new market for a differential industrial purpose had opened, increasing the Swiss farmers WOSR production portfolio. Its production started with the OP-line variety Splendor. It was replaced in 2007 by the OP-line V141OL, then by the Ogura Hybrid V280OL in 2012. A new hybrid variety, V316OL, was registered in 2014 and will be grown in 2015. Its average yield is 20% higher than V280OL. Yield and quality were alternatively improved by the genetic progress of new varieties. The average yield increased from 2.5 t/ha in 2004 to 3.3 t/ha in 2014. Important progress was also observed in relation to oil quality. Linolenic acid content decreased from 3.5% on average in 2009, to 2.5% in 2014. Oleic acid increased to 79% between 2007 and 2012 (V141OL), then decreased to 77% with V280OL from 2012 to 2014. This evolution, yielding a more heat-stable oil, is not only due to a varietal progress and environmental consequences, but it is also the result of a successful partnership among all the players of the production chain. Farmers are well aware of the importance of good agricultural practices to produce a high-quality HOLL oil, with low linolenic acid content (long rotation, soil tillage to limit volunteers, avoidance of conventional-HOLL seed mixing, distance between HOLL and conventional WOSR fields, etc., Baux et al. 2013). A cost/benefit analysis, based on gross margin evolution for each sector in the value chain, showed that the economic benefits related to the HOLL WOSR segment were significant and were shared by all the players along the value chain (Pellet 2011).

**Conclusions:** The development of a new type of WOSR allowed for the establishment of an additional WOSR market in a relatively short period. To satisfy producers as well as the industry, continuous efforts are made to optimize quality in terms of yield and oil quality. New varieties are tested each year and efficient crop management is investigated to secure this new market.

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# Major proteins of canola have distinct structural and physico-chemical properties

**Background:** Commercial interest in canola protein for food use has resurfaced due to the increased demand for plant proteins. Besides the nutritional indicators, the functional performances which directly relate to the protein composition determine positioning of canola protein products in the food protein market. Protein products recovered from de-oiled canola contains storage and structural proteins of the seed. Crucifer seed storage proteins cruciferin (CRU, 11S) and napin (NAP, 2S) compose the majority of recoverable proteins from canola. Limited information (Wu & Muir, 2008) is available on the structural basis of functional potential and performance of canola CRU and NAP. Most of the canola protein isolates described in scientific literature are CRU and NAP mixtures in various proportions.

**Objectives:** Comparative investigation of structural and physico-chemical properties of 11S and 2S storage proteins of canola seeds to predict functional performances.

**Methods:** *Brassica napus* seeds produced in greenhouse conditions were de-oiled with hexanes and meal protein was extracted at pH 8.5 using Tris-PO<sub>4</sub> buffer containing 0.75 M NaCl. Cruciferin and napin isolation and purification was according to Berot et al. (2005). Structural and physico-chemical property characterization of these two proteins was by electrophoresis (native, 1D and 2D) and spectroscopic (UV-CD, fluorescence and FT-IR) methods. Amino acid composition was determined according to official method of analysis.

**Results:** CRU and NAP obtained from this purification process had >98% protein based on total N. Hexameric nature of CRU was confirmed and it was consisted of polypeptides ranging from 56-19 kDa. Un-dissociated NAP had molecular mass of 13 kDa that resulted in 10 and 7 kDa polypeptides upon S-S bond reduction. Presence of isoforms was evident for both CRU and NAP. Amino acid profile and isoelectric focusing profile confirmed the basic nature of NAP and neutral nature of CRU. Napin showed shallow endothermic transition with peak denaturation temperature at 96°C and Cruciferin showed at 90°C. Cruciferin had very low solubility between pH 3 and 8 but become nearly 90% soluble at pH 2 and 10 while napin showed 100% solubility between pH 3 and 9 with a slight depression at pH 7.4. Secondary structure of CRU was sensitive to pH change while NAP was not. Neutral salts at 0.2 and 0.5 M concentration enhanced solubility of CRU across pH 3 to 10 but solubility of NAP was affected negatively.

**Conclusions:** Major storage proteins of canola have quite distinct structures and molecular characteristics. Therefore protein products obtained from canola will have different properties and functionalities based on the abundance of these two proteins. Separate recovery of these two protein types will enable to make use of their inherent value more effectively.

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## Nitrogen value for ruminants of regular solvent-extracted rapeseed meal

**Background:** The solvent-extracted rapeseed meal (RSM), which is produced in France, presents a certain between- or within-factory variability. This variability can modify the nitrogen value for ruminants.

**Objectives:** The aim of the study realized in 2012 was to compare in sacco nitrogen effective degradability (NED) and in vitro nitrogen enzymatic degradability (DE1) of different RSM in order to verify the relation between these criteria used for the prediction of nitrogen value for ruminants.

**Methods:** In sacco and in vitro measurements were conducted on 15 samples of RSM, quite representative of the existing variability studied with 35 samples of RSM collected previously by Chapoutot et al. (2011). In sacco measurements were done as described by Michalet-Doreau et al. (1987) with a double latin square including 3 cows and 6 replicates. The data measured in this study have been integrated in the new "Systali" model described by Chapoutot et al. (2013) to calculate the new nitrogen values of RSM.

**Results:** The present values of nitrogen enzymatic degradability (DE1) are much lower than those obtained for 2 decades, which were exploited for the elaboration of the prediction model of nitrogen effective degradability (NED) nowadays used in France. The reduction of the DE1 values of RSM is certainly related to an increase in the temperature of the treatments used in the extraction processes. The results of in sacco measurements allowed to confirm the NED values proposed in the Tables INRA (2007) for this feed and to validate the accuracy of the prediction model of NED. Moreover, these new results lead to increase the precision of the estimation of NED from current DE1 values. The data measured in this study have been integrated in the new "Systali" model to calculate the new nitrogen values of RSM. Compared to INRA 2007 ones, the new Systali "Table" values are modified, with a higher PDIN/PDIE ratio (higher PDIN value and lower PDIE value). A simulation was done with the new calculator "Systool" in order to quantify the effect of the inclusion of RSM in maize silage-based diets for dairy cows upon its real nitrogen values. The digestive interactions, much more precisely taken into account in the "Systali" model, tend to slightly increase the nitrogen "Ration" values of RSM (especially the PDIN value) compared to the "Table" ones.

**Conclusions:** These results do not call into question the model validity of the prediction of NED with DE1. The mean values of the 15 samples of this study are very closed to those of the INRA 2007 ones.

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## Collaborative inter-laboratory study on glucosinolates analysis for ISO 9167-1 revision

**Background:** Analyzing glucosinolates (GSL) in rapeseed and other *Brassicaceae* remains crucial to ensure the quality of the seeds for certification, trade and the meal for animal feeding. Reverse phase liquid chromatography of the desulfo-GSL was standardized (ISO 9167-1, 1995) as the reference method and revision became necessary because of new safety and efficiency requirements.

**Objectives:** The aim was mainly to substitute the methanol, considered toxic, used for the extraction procedure, extend the scope to other *Brassicaceae*, test a rapid isocratic mode of elution, and finally, determine the precision data of the revised method.

**Materials and methods:** Six samples of seeds (3) and meal (3) from France (3) and Canada (3) with various GSL contents (from 1.5 to 150  $\mu\text{mol/g}$ ) were prepared by appropriate homogenization-division, and sent to each of the eighteen laboratories from thirteen countries which agreed to participate to the collaborative. Two samples were *Brassica juncea* with low and high GSL contents. Duplicate analyses were required with gradient and isocratic HPLC elution modes, the gradient elution (reference method) being applied in priority. The statistical treatment was performed according to ISO 5725-2 with Cochran and Grubbs tests to detect stragglers and outliers.

**Results:** Eighteen laboratories sent results for the gradient elution mode and only six for the isocratic one. The ring-test was carried out without noticeable difficulties for the gradient method, especially for the revised operating mode part (extraction by ethanol 50%, no replicate, purification method for sulfatase). Some problems still remained regarding the availability of the internal standard glucotropaeolin required for the isocratic elution.

For the gradient mode, the relative standard deviations of repeatability (RSDr) and reproducibility (RSDR) ranges were 1.2 - 6.1% and 9.2 - 21.0%, respectively. For the isocratic mode, the ranges of RSDr and RSDR were 0.4 - 3.1% and 12 - 59.0%, respectively. The relative bias between results from the two elution modes ranged from -1.4 to 10.3% for the five samples with GSL contents higher than 5  $\mu\text{mol/g}$ , and 41% for the sample containing less than 2,5  $\mu\text{mol/g}$  GSL. The number of retained laboratories for the isocratic mode did not meet the ISO requirements (6 or 5 vs. 8) for a collaborative study.

**Conclusions:** Comparison with previous ring-tests performed in 1988 and 1992 indicated that precision data obtained for the present study were similar, even better in the tested range of GSL content than previous precision data. The added data for the mustard samples (*B. juncea*) allow now to include this type of seed in the scope of the revised standard. Although results from both elution modes were similar, the isocratic mode should be only included in the informative annex, the gradient elution mode being the reference method.

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# Glucosinolates and by-products in rapeseed meal related to hydrothermal processing

**Background:** For safety reasons, rapeseed meal (RSM) is usually desolventized with strong hydrothermal treatments, leading to various levels of residual glucosinolates (GSL), and protein solubility. The RSM nutritional quality may be then lowered for monogastrics, due to GSL breakdown products whose reliable and “easy to use” indicators lack.

**Objectives:** The study aims to propose and test methods for identifying and quantifying breakdown products from GSL, and myrosinase activity. The relationship between hydro-thermal treatment of RSM and the routes of the GSL degradation should also be helpful to predict the anti-nutritional potential of the meal in the diet.

**Materials and methods:** Preparation of material to be tested : 1) GSL extracts were purified from seeds of *Brassica napus*, *B. campestris* and *Crambe abyssinica* leading to batches with different GSL profiles. 2) 2 batches of 1.5 kg rapeseed seeds (one of them was previously blanched with steam to inactivate the myrosinase) were pressed and deoiled with hexane then desolventized at low temperature. The two RSM batches were treated by either dry or wet toasting at 110°C from 15 to 90 min by the means of a bench-cooker, to promote different routes for GSL breakdown.

**Analysis:** GSL according to ISO 9167-1, isothiocyanates (ITC), nitriles and oxazolidine-2-thiones (OT) by GC-MS and GC-FID, myrosinase activity by enzymatic assay and spectrophotometry. Methods were applied on purified extract of GSL first, then on the RSM samples collected in the bench-cooker after 30 min of treatment.

**Results:** 1) Methods of analysis for GSL, OT and ITC were successfully tested on purified GSL extracts under various conditions to breakdown GSL into OT, ITC and nitriles. Recoveries and balance were consistent with the reaction stoichiometry. When purified GSL were added to RSM, recoveries remained at high level for GSL (90-100%), but decreased for OT (40%), nitrile and ITC (10%). The relationship observed between recovery and quantity of added ITC indicated a matrix effect, probably due to interactions with amino-acids.

2) GSL analysis of the eight RSM samples processed on the bench-cooker did not show any effect of the blanching. The effect of the toasting on GSL breakdown was higher when steam was applied (at 30 min: 45% vs. 27%). Nitriles were detected in the blanched RSM while only ITC were detected in the no blanched RSM. These observations were in agreement with the literature data on the GSL degradation and the routes with or without myrosinase activity.

**Conclusion:** Although nitriles and ITC were detected at a low level, these results showed that the GSL degradation route in a processed RSM could be determined by chemical analysis. The residual GSL content and myrosinase activity can be accurately determined and the quantity of ITC or nitriles produced could be then predicted. Nevertheless, as ITC can be linked to amino-acids, further studies are necessary to evaluate their bio-availability, their actual anti-nutritional effects and their impact on the protein quality. The latter can also be directly reduced by the hydrothermal treatment applied to the RSM.

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# Evaluation of ethanol and 2-propanol for rapeseed oil extraction

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**Background:** Oil extraction from rapeseed or sunflower involves preparation of the seeds including conditioning and pressing, solvent extraction and solvent elimination from oil and residue. Hexane is commonly used by industry because of high efficiency and specificity for oil extraction and low energy required for its removal from miscella and meal. Nevertheless, hexane has drawbacks like high flammability, dangerousness for health and environment deleterious effects and then, makes relevant the research for alternative solvent. Ethanol (EtOH) and 2-propanol (2POH) because of their lower toxicity and solvent properties could be candidates.

**Objectives:** EtOH and 2POH were compared to hexane for rapeseed oil extraction. Yield and specificity of the oil extraction were determined and feasibility of EtOH and 2POH recycling in continuous extractors was evaluated.

**Material and methods:** The experiments were performed with a 6 L extractor to simulate a cross flow extraction on a multistage extractor. 2 kg of rapeseed press cake (19% oil, 9% moisture) was extracted by circulating 5 L of solvent during 15 min and the operation was repeated six times with renewing the solvent (EtOH/water (90.4/9.6), 2POH/water (85.2/14.8) and technical hexane). The miscellas from EtOH and 2POH were then cooled to recover the extracted oil by dephasing and coalescence.

**Results:** The oil residue in meal was less than 2% with each of the three solvents. The extraction efficiency was strongly correlated with their hydrophobicity: hexane > 2POH > EtOH and the necessary number of washings for each solvent was 4, 5 and 7 respectively. EtOH and 2POH allowed extracting polar compounds such as glucosinolates (40 - 50% decrease). Tocopherols were better extracted with EtOH (1930 ppm) than with 2POH (818 ppm) or hexane (743 ppm). Due to the extraction of other polar compounds, the protein content of the meal was increased by 3 (2POH) and 4 points (EtOH). Recovery of the oil from the miscella with EtOH and 2POH was performed (yield around 75%) by dephasing at 4°C. Crude oil from alcohols has very low phosphorus content (12 ppm vs. 554 ppm for hexane). In contrast, the enrichment in water after several cycles due to the moisture of the extracted material was critical, particularly for EtOH which properties to extract oil were altered. The solvent also required to be purified to separate and recover polar compounds.

**Conclusion:** Compared to hexane, EtOH and 2POH needed more volume and contact time to completely extract the oil. Partial extraction of polar compounds was observed, that could lead to increase the meal quality (more protein and less glucosinolates) but requires the solvent to be purified. The moisture content in the material and the solvent should be maintained at a low level to make easier the solvent recycling. Greater tolerance for water and improved extraction kinetics were observed with 2POH, but EtOH provided more promising quality improvement for oil and meal and should receive a better acceptance by consumers.

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# Composition of kernel and hull fractions obtained from rapeseed dehulling

**Background:** Although dehulling oilseeds enhances the meal quality, this process was rarely applied at industrial scale to rapeseed because of the oil-losses in hulls and the possible effect of the glucosinolates concentration in dehulled meal. The knowledge of the bio-chemical composition of both hulls and kernels from current rapeseed cultivars would notably help to optimize the dehulling level to be economically profitable.

**Objectives:** This work aims to characterize the bio-chemical composition of the whole rapeseed seed, the pure kernel and hull fractions, and to study the components variability with various cultivars and cultivation areas.

**Methods:** Six rapeseed cultivars were grown in both Cher and Charente-Maritime areas in France. Dehulling was performed in a vertical-conical impactor and based on the projection by the centrifugal force of the seed to the internal wall of the machine (CETIOM dehuller). Then, kernels and hulls fractions were separated by aspiration and sieving. Seeds were frozen (24 h; -20°C) before dehulling to obtain pure hull fractions. They were previously dried (2h, 60°C), then frozen (24 h; -20°C) to obtain the pure kernel fractions. Contents of water, oil, proteins, ash, fiber, crude cellulose and glucosinolates were measured on whole seeds, pure kernel and hull fractions. ANOVA was used to determine correlations between components.

**Results:** The hull mass fractions ranged from 16.8 to 21.2% of the seed mass. The proportions of the various constituents of the seed located in the hulls were: oil: 2.9%; protein: 11.2%, ash: 30%, Neutral Detergent Fiber (NDF): 73%, Acidic Detergent Fiber (ADF): 80%, lignin: 95%, glucosinolates: 3.7%. The hulls were less rich in oil ( $8.4\% \pm 1.6\%$ ) than usually reported (10.6-16.4%).

The dried deoiled pure kernel fraction contained 47.8% of proteins, 10.8% of NDF, 6.6% of ADF, 0.5% of ADL, 5.5% of ashes and the major part of the glucosinolates (on average 93.4% of the whole seed). A strong negative correlation was observed between the proteins content of the hulls and their fibers component ( $R = -0.86, -0.89, -0.70$  and  $-0.78$  for the NDF, ADF, ADL and Crude Fiber, respectively). The ANOVA revealed no significant relationship between cultivar, growing conditions and hull content of the seeds, although the oil content and the protein content of the fractions were significantly affected by cultivar and to a lesser extent by the location of the crops.

**Conclusion:** The complete removal of rapeseed hulls could result in high protein meal, which content corresponds to 125 % of the proteins of whole seed meal. This high protein meal would have 130% of the glucosinolates of the non-dehulled meal but only 37% of its NDF, 30% of its ADF and 5% of its ADL. Ashes would remain equal while crude fiber would be decreased to 37% of the non-dehulled meal.

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## Solid/liquid expression behavior of rapeseed during discontinuous pressing

**Background:** Solid-liquid expression or pressing is a unit operation in which liquid is separated from solid-liquid mixture by mechanical compression. This technology is widely used for rapeseed oil extraction and its control is a major issue in the efficiency of the crushing process since the characteristics of the expeller cake such as the mechanical strength and the porosity, may affect the efficacy of the solvent percolation. Although pressing is mainly performed in oil mills, on continuous flow screw presses, the study of discontinuous pressing is of interest to better understand the relationship between the pressing efficiency, the seed quality, the pretreatments and pressing parameters.

**Objectives:** As the mechanism of solid/liquid expression from rapeseed seeds during pressing is very complex, this work is devoted to better understand it by modelling the compression behavior of the seeds.

**Methods:** The impacts of seed pretreatments (dehulling, crushing/flaking and cooking) and pressing parameters (pressure, temperature) on the solid-liquid expression behavior of rapeseed were studied based on the theory of filtration/consolidation. This theory gives a comprehensive approach for description of liquid flow inside compressible porous materials based on the analogy with Fick's diffusion theory. Indeed, the seed pressability is characterized by a consolidation coefficient  $b$  ( $m^2.s^{-1}$ ). In this work, the applicability of a semi-empirical model to calculate this coefficient was investigated. Rape seeds were first pretreated (cooking, flaking) under different conditions. Samples (300 g) of treated or untreated seeds were then compressed using a hydraulic press for 60 min. The pressure and the temperature were varied from 20 to 150 bar and from 20 to 110°C, respectively. For each experiment, the oil yield was determined, the thickness of the press cake was recorded during pressing and the corresponding consolidation coefficient was calculated.

**Results:** Results showed that the highest oil yield ( $\approx 70\%$ ) was obtained by pressing flaked and cooked seeds (90°C during 30 min) at 100 bar. On the other hand, it was shown that the employed model allowed the determination of the consolidation coefficient only for pre-treated seeds (cooked, flaked). The compression behavior of untreated seeds cannot be described by filtration-consolidation behavior. For treated seeds, the consolidation coefficient increased with pressure and temperature. For example, it increased from  $5 \cdot 10^{-7} m^2.s^{-1}$  to  $3 \cdot 10^{-6} m^2.s^{-1}$  when the pressing temperature rose from 20°C to 110°C. In fact, the faster was the cake deformation, the higher was the consolidation coefficient. Experimental and model data adjustment showed that the model reasonably well approximated the experimental data for a consolidation behavior index equal to 0.5. This result reflected that treated seeds presented creep characteristics and, both primary and secondary consolidations of tissue occurred.

**Conclusion:** The obtained results showed significant effects of seed preparation (crushing/flaking and cooking) and pressing parameters on the pressing efficiency and the consolidation behavior. In the case of prepared seeds, the proposed semi-empirical model allowed a good prediction of the consolidation kinetics without needing to perform long and expensive experiments. The results are profitable to extend the research on continuous pressing.

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# Chemical composition and nutritive value of meals from yellow-seeded canola

**Background:** *Brassica napus* is the most commonly grown canola species in Canada. In recent years, breeding attempts to increase oil in the seed and reduce fibre content in the meal, lead to the production of yellow-seeded *B. napus* canola and canola-quality *B. juncea*. Earlier research from our laboratory has demonstrated superior quality characteristics (i.e., increased protein and sucrose, and reduced dietary fiber contents) of these meals in comparison with conventional *B. napus* black.

**Objectives:** To evaluate the chemical and nutritive composition of meals derived from yellow-seeded *B. napus* and *B. juncea* canola and to determine amino acid digestibility and available energy contents needed for diet formulations in a subsequent growth performance study with broiler chickens.

**Methods:** Canola meals for this study were produced using a large-scale, pre-press solvent extraction process. Apparent metabolizable energy (AMEn) and standardized ileal amino acid digestibility (SIAAD) of *B. napus* yellow, *B. juncea*, and the conventional meal were determined with broiler chickens from 14 to 19 d of age (AMEn assay), or from 14 to 21 d of age (SIAAD assay) using 6 pens of 6 birds each per treatment. Birds were fed either basal diet (control group) or the basal diet supplemented with 30% of canola meals for AMEn, and test ingredient as a sole source of protein for SIAAD evaluation. The nutritive value of canola meals was further validated using 7 pens of 50 broiler chickens per treatment. Birds were fed wheat/corn/soybean meal-based diets containing 15% of canola meals in the starter (1-10d), grower (11-24d), and finisher (25-36d) phases of the experiment.

**Results:** In comparison with the conventional meal, yellow-seeded *B. napus* and *B. juncea* contained (DM basis) more crude protein (43.4 and 47.2 vs. 41.1%), more sucrose (10.1 and 8.0 vs. 6.6%), and less total dietary fiber (29.8 and 28.9 vs. 35.0%), respectively. The highest content of all essential amino acids (except cysteine) was observed in *B. juncea* meal. The AMEn and SIAAD values for *B. napus* yellow, *B. juncea* canola, and the conventional *B. napus* black were 1865, 2092 and 1902 kcal/kg DM, and 82.5, 83.2, and 81.8%, respectively. Enzyme (multicarbonylase) supplementation resulted in AMEn values of 2131, 2264 and 1851 kcal/kg DM, respectively. In the growth performance study, BWG averaged 2.32, 2.30, 2.19, and 2.31 kg for the control, *B. napus* black, *B. napus* yellow and *B. juncea* meals, respectively, and no significant difference in FCR between the control and the diets containing canola meals were observed indicating that all types of canola meal could be used effectively and replace SBM in broiler chicken rations.

**Conclusion:** It would appear evident that breeding for low-fiber canola would result in quantitative rather than qualitative changes as evidenced by increased oil, protein, and sucrose contents and decreased fiber content in the seed. Canola meal could be used effectively in broiler chicken rations at 15% in all 3 phases of growth when diets are formulated based on digestible amino acids and available energy contents.



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# Changes in seed quality during maturation of double-low winter oilseed rape

**Background:** Oilseed rape is an important crop in China. Over 1.000 million tonnes of rapeseed are produced annually for the extraction of edible oil and the meal is a useful source of protein in livestock feeds. In general, Production of high quality seeds depends upon the appropriate time of harvest. However, it is rainy during harvest of oilseed in China, which could result in yield losses. In order to avoid the rainy season, seed should be harvest as soon as possible, when physiological maturity has been reached. This study was done to verify the development stage and to define the optimum harvest date by analysis of changes of seed quality.

**Objectives:** Five double-low conventional varieties and 3 double-low hybrids varieties were used. The samples were collected continuously once per five days and started tentatively on early May.

**Methods:** The materials were sown in multi-point areas in two years. NIR analysis of oil content and glucosinolate content was carried out using a Foss NIR Systems Series-5000. The fatty acid compositions were determined by gas chromatography.

**Results:** The glucosinolate content remained stable during maturation. However, there was a significant increase in oil content until May 16, and then no change. The change of the fatty acid components in seed was very different with each other. The content of erucic acid, stearic acid and oleic acid remained stable during maturation. There was a significant reduction of palmitic acid, but there was a significant increase between linoleic acid and linolenic acid.

**Conclusions:** There are some changes of the oil content in the different year because of the variable climates, but the oil content would reach the highest value in May 16-18 each year, and it is just time to physiological maturity (Li et al, 2009). According to fatty acid composition, seeds could be harvested after physiological maturity, but it is the optimum harvest period after the ripening stage.

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## Oil quality traits in Canola. Understanding and manipulating saturated fatty acid content

**Background:** Although Canola oil is already a healthy oil, considered low in saturated fatty acids, further reduction of saturated fatty acid content is a trait of interest in Canola breeding. To better understand saturated fatty acid biosynthesis in the developing canola seed we generated plants with increased total saturate content through micro-RNAi mediated gene silencing and cDNA overexpression. We also took a non-GM approach to silence individual genes encoding enzymes of fatty acid biosynthesis, generating a series of plants with reduced saturate content.

**Results:** Data indicates that targeting specific members of gene families encoding enzymes of primary metabolism can result in plants with a heritable phenotype of reduced seed saturated fatty acid content with no obvious detrimental effects. Plants producing seed containing more than 40% saturated fatty acid content were comparable to control plants in growth and seed yield, but were slow to establish at low temperature.

**Summary:** A number of examples will be presented with altered levels of seed oil saturated fatty acid content. The potential for further development of new seed quality traits will be discussed.

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# Performances of rapeseed meal in dairy farms in France

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**Background:** Zootechnical and economic results registered in farms confirm the interest of rapeseed meal (RSM) in dairy rations. However, the generalization of its use may be limited due to a number of questions that persists about this raw by-product.

**Objectives:** The objectives were to measure the influence of high levels of dietary RSM on the production and reproduction performances and the sanitary status of dairy herds and to identify the limitations, motivations and interests of the incorporation of RSM in Haute-Marne area.

**Methods:** Two homogeneous groups of 42 farms on maize silage system composed of matched pairs, were made by multivariate analysis. For the RSM group, the ration included at least 4 raw kg/d of RSM. For the other group (No RSM) diet supplementation was achieved by other nitrogen correctors. Animal performances were recorded for 3 months after herds' selection during the month preceding the study. The measured variables were: milk production per cow, fat and protein contents, artificial insemination (AI) success, leucocytes and mastitis. The feed cost and feed margin were calculated using purchase prices of concentrates and by-products, and standardized prices for fodder (100 € / TMS for corn silage). The data were processed by variance analysis.

The sociological study was based on the realization of semi-structured interviews to collect the reasons and motivations of farmers to use, or not, RSM. Interviews were also applied to professionals that work with farmers to find out what is RSM image in farms.

**Results:** It appeared that the addition of a large amount of RSM ( $4.8 \pm 0.7$  kg /dairy cow/d of RSM on a total of  $5.2 \pm 0.7$  kg /dairy cow/d of nitrogen corrector) in the ration of RSM group, compared to No RSM group, did not significantly changed the raw milk production ( $-0.2$  kg/d) or fat content ( $-0.2$  g / kg). However, it significantly increased the milk protein content ( $+0.3$  g/kg,  $P < 0.05$ ), although the intake of the two groups was nearly identical. Reproductive performances, with the success rate of first AI on 3-month study ( $+1.1\%$  success) and the health status of the udder (mammary cell count and % of infected cows /month) were not significantly modified. On the economic front, farms of RSM group compared to the Not RSM group, had a lower ration cost of  $0.48$  € /cow/day ( $P < 0.001$ ), with a difference of  $-15.9$  € /1000L of milk, and presented a higher feed margin of  $+0.42$  € /cow/day ( $P < 0.05$ ) with a saving of  $+17$  € /1000L.

RSM, as a local and non GMO raw material with attractive price, has a good image among breeders and professionals. However, it is perceived as a less "noble" product compared to soybean meal. The sociological study reveals that feed companies are not particularly favorable to rapeseed meal.

**Conclusions:** This study confirms that significant amounts of rapeseed meal can be introduced into dairy rations without affecting performances and allow an improvement of production costs of the dairy sector.

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## Effect of rapeseed meal on milk and cheese quality

**Background:** In France, about 2.3 million tons of rapeseed meal are used in feedstuff and 60% of them for ruminants. Rapeseed meal is actually the first meal included in dairy cows diets because of its economical interest. Nevertheless we observed a lack of references on the ability of milk for cheese production.

**Objectives:** In that context of replacing soybean by rapeseed meal in dairy cows diets, a trial was done in order to compare the quality of milk and cheese of cows fed with soybean or rapeseed meal.

**Methods:** 2 groups of 7 Holstein cows were fed with whole diets composed of maize silage, sorghum and rapeseed or soybean meal for 5 weeks followed by 2 weeks of transition and 5 weeks of inversed diets. Milk of each diet was collected for cheese fabrication.

**Results:** With the use of rapeseed meal replacing soybean meal, cows produced as much milk and this milk was richer in proteins ( $P<0.01$ ). As well, a significant increase in milk casein content and casein/protein ratio was observed. It was higher than the one observed by Martineau et al. (2013). Rapeseed meal induced a decrease in total and soluble calcium contents of milk without affecting colloidal calcium and phosphorus contents, and induced an increase in soluble phosphorus. Rapeseed meal decreased saturated fatty acids percentage and increased oleic and linolenic acids.

With rapeseed meal, milk coagulation and curd firming times were longer as mentioned by Mietton et al. (2004). Curd firmness and cheese yield decreased with poorer retention of fat in the curd, without any effect on physico-chemical composition of cheese at the end of ripening. Only a few volatile compounds (12.5%) were significantly affected by the diet.

Rapeseed meal significantly enhanced the concentration of ketones and decreased the concentration of esters, sulfur compounds and furans. The diet did not induce rheological differences between cheeses but their texture was slightly more grainy and less sticky with rapeseed meal. The salty and pungent tastes, the "little sour milk" and spicy aromas were greater ( $P<0.05$ ) with rapeseed meal and broth, egg yolk ( $P<0.05$ ), hazelnut and butter aromas ( $P<0.10$ ) were greater with soybean meal.

**Conclusions:** These first results show that rapeseed meal improves the production of milk proteins but modifies the cheese production probably in relation with the increase of casein/protein ratio. Mineral correction of diets or technological modifications of cheese production have to be tested in order to try to correct times of coagulation.

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## Towards understanding the genetics controlling a low saturated fat trait in *Brassica napus* and transfer into commercial inbred canola lines

**Background:** Canola oil from *Brassica napus* is promoted as healthy edible oil since it contains the lowest level of saturated fatty acids (SFA) of any vegetable oil. A shift in cultivation from Polish canola to Argentine canola in the 1990s resulted in an increase in total SFA content (Rakow and Olson 2010; Raney et al. 1999). For the past 13 years, AAFC has been developing stable lines that are low (<5%) in SFA content using a combination of methodologies including interspecific crossing and mutagenesis breeding strategies (Rakow and Olson 2010; Raney et al. 1999).

**Objectives:** 1) Investigate genetic control of total and major SFA components: dominance behavior, maternal inheritance and population distribution of Palmitic acid (16:0) and Stearic acid (18:0). 2) Examine the stability of the low SFA trait across multiple environments within segregating populations. 3) Identify highly productive lines containing the low SFA or lower SFA to use in hybrid combinations or elite crosses.

**Methods:** The low saturate (<4.5%) line was crossed with 10 advanced breeding lines. In 2 seasons, open-pollinated and/or self-pollinated F1:2 and F2:3 seed was harvested from 3 and 2 reciprocal cross combinations, respectively and F1:2 seed from 7 additional one-way crosses. From six of the cross-combinations, doubled haploid (DH) populations were developed from reciprocal F1s. A total of 545 DH lines were evaluated at two locations in 2013 and 388 in 2014. Harvested seed from all trials were analyzed for fatty acid profile including all SFA components using gas chromatography.

**Results:** Based on fatty acid profile analysis of bulked harvested F1:2 seed, the trait appears to be incompletely dominant in all 10 cross combinations. There was little evidence of maternal effect in F2:3 seed from 2 reciprocal cross combinations; however, there was strong evidence of maternal inheritance for 18:0 and total SFA in the F1:2 seed and in the DH populations. The frequency distributions of the F2:3 populations were continuous and normally distributed; however, distributions of all DH populations were bimodal for palmitic acid. Four of the DH populations fit a 1:1 segregation model for palmitic acid suggesting control by a single major gene. Frequency distributions of DH lines were continuous for 18:0 and total SFA. Transgressive segregants for total SFA lower than the trait parent were not present in any population; however, lines with lower SFA with improved agronomics compared to the trait parent were identified. Correlations between sites in 2013 were high (0.90 to 0.99) for total SFA and components across DH populations.

**Conclusions:** Based on these results, it is expected that the low SFA mutations will be useful for overall reduction of SFA within hybrid breeding programs where the trait is only incorporated into one hybrid parent, but reduction of saturates to less than 5% will require the trait to be introgressed into both hybrid parents.

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POSTERS THEME C

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## Digestibility additivity in growing pigs of phosphorus from biofuel by-products

**Background:** Controlling phosphorus releases is an important environmental issue for swine production. Feed remains the most interesting way to limit its excretion by pigs. The use of Biofuel by-products from wheat and rapeseed has increased in France, but phosphorus digestibility data are little or unknown, even less the additivity when mixed in a feed.

**Objectives:** The aim was the measurement of the true digestibility of phosphorus in rapeseed meal (RSM) and bioethanol by-products from wheat (BBP), and the evaluation of the additivity for this criterion in a two by-products mixture.

**Methods:** The true faecal digestibility (tdP) and the phosphorus retention coefficient (rcP) were measured for two bioethanol by-products (BBP1 and BBP2) and for one biodiesel by-product (RSM) included at 25%, and a mixture of 12.5% BBP1 and 12.5% of RSM (BBP1/RSM) in semi synthetic feeds on five pigs per treatment for 5 days of collection after 14 days of adaptation.

**Results:** The tdP of BBP 1 and BBP 2 was similar (NS) and high (50.4 and 53.1%), but the release of urinary P was important, probably due to low dietary Ca, that induced a drop of rcP (28.7 and 34.3%). The RSM had very close tdP (33.1%) and rcP (32.3%). The association of BBP1 and RSM led to an intermediary tdP (43.7%). As the three diets were significantly different from each other ( $p < 0.001$ ), this result was consistent with the hypothesis of additivity of tdP. The rcP was improved ( $p < 0.01$ ) in the BBP1/RSM (38.4%) compared to the others, probably by balancing the Ca/Pd ratio.

**Conclusions:** True digestibility of phosphorus was evaluated in two bioethanol by-products ( $\approx 50\%$ ) and in a rapeseed meal ( $\approx 33\%$ ). The additivity of phosphorus digestibility of a bioethanol by-product and a rapeseed meal when associated in a same feed was confirmed.

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# Changes in canola seed protein structure and properties during pre-press solvent extraction process

**Background:** Canola (*Brassica napus*) meal of pre-press solvent extraction (PSE) is dark, has lower protein solubility and in vitro digestibility than laboratory prepared meal (Garla et al., 1994). Protein is the most valuable fraction of canola meal but protein recovery from PSE meal is difficult and results in protein products having poor functionalities (Pastuszewska et al., 2003). Including all the intermediate steps, pressure, temperature, and solvent environment changes associated with PSE may have direct effect on seed constituents leading to physico-chemical changes of meal components as evidenced by the altered nutritional value of PSE meal (Mustafa et al., 2000).

**Objectives:** Investigate the changes in the physico-chemical properties and structural details of protein fraction of canola seed during PSE in order to find out the critical processing step/s that affects protein structure and properties.

**Methods:** Samples from commercial PSE operation were obtained at 5 different stages; 1) feeder, 2) cooker (98-100°C), 3) press (60-100°C), 4) solvent extractor (60-70°C with hexanes), and 5) desolventizer (82°C upper and 112°C lower deck) from three different processing cycles spanned in a 3-month period. Total protein, total oil, protein dispersibility index (PDI), protein solubility at pH 4, 7 and 10, FT-IR spectral characteristics, in vitro protein digestibility with pepsin and pancreatin, available lysine content and total free sugar contents were determined.

**Results:** The oil-free basis protein content of the meal was at 38.6–41.8% level as the oil extraction progressed. PDI values of the meal were reduced by ~30% after cooking and ~65% upon desolventizing. Complete loss of pH 4 soluble protein fraction (~17.5% in seed) after desolventizing indicated that napin (2S) protein became insoluble. Insoluble protein content started to increase after the cooking step as soluble protein content at pH 7 and 10 was reduced by 20 and 39%, respectively. Desolventizing caused up to 70% reduction in pH 10-soluble protein content. Increases in the beta-sheet and random coil component levels and the changes in the amide I, amide II, -PO<sub>3</sub> and C=O related functional areas of FT-IR spectra indicated destabilization of proteins at secondary structure level. Changes in the levels of available lysine and free sugar contents were evident but not the in vitro protein digestibility values.

**Conclusions:** Processing steps that involve temperature >70°C contributed to the changes in the properties and structural components of canola proteins indicating modifications in the proteins and their association with other seed constituents.

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# Seed coat color and oil content in *Brassica rapa* populations

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**Background:** *Brassica rapa* (*B. rapa*) is an important vegetable and oilseed worldwide. Most *Brassica* species are black-seeded, such as Chinese cabbage in *B. rapa*. However, there are natural yellow-seeded mutations such as yellow sarson in *B. rapa*. Yellow seeds are associated with significantly thinner seed coat, reduced cell size in all seed coat tissue and larger embryos. These characteristics lead to a low hull proportion and high oil and protein content in the seed (Stringam et al. 1974; Rahman et al. 2001).

**Objectives:** In *Brassica* breeding programs, seed coat color and oil content are valuable traits. In the current study, seed oil content of two *B. rapa* populations were analyzed for the relationship between seed coat color and oil content.

**Methods:** Two hundred recombinant inbred lines (RILs) have been developed using two crosses (yellow sarson x turnip rape [BU] and yellow sarson x Chinese cabbage [SR]). Each of the 200 RIL were analyzed for oil content and seed color. Seed color was classified into yellow, light yellow, brown and dark brown. Near-infrared spectroscopy (NIR) and solvent extraction methods were used to collect oil content data.

**Results:** Using the NIR method in the BU population, oil content of yellow-seeded RILs increased by 1.91% and 2.56% when compared to brown-seeded and dark brown-seeded RILs, respectively. When using the solvent extraction method, oil content of yellow-seeded RILs increased by 3.01% and 2.86% when compared to brown-seeded and dark brown-seeded RILs, respectively. In the SR population using the NIR method, oil content of yellow-seeded RILs showed an increase of 3.58% and 1.4% when compared to brown-seeded and dark brown-seeded RILs, respectively. Under the solvent extraction method, oil content of yellow-seeded RILs showed an increase of 3.3% and 0.85% when compared to brown-seeded and dark brown-seeded RILs, respectively.

**Conclusions:** NIR and solvent extraction results showed that yellow-seeded *B. rapa* had higher oil content compared with brown and dark brown seeds. Since *B. rapa* is one of the parental species of *Brassica napus*, yellow-seeded *B. rapa* progenies can be integrated into canola breeding for higher oil content.

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# Biochemical properties of *Brassica napus* diacylglycerol acyltransferase 1

**Background:** Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of diacylglycerol to form triacylglycerol (TAG), which is the main component of seed oil. In *Brassica napus*, the level of DGAT activity has a substantial effect on the flow of carbon into seed oil (Weselake et al., 2008). Although membrane-bound DGATs have been studied for several decades, their mode of action and regulation remains poorly understood due to difficulties associated with purification.

**Objectives:** Studying the properties of DGAT1, a key enzyme in oil biosynthesis, will increase our understanding of the regulation of seed oil formation.

**Methods:** Recombinant *B. napus* DGAT1 (BnaC.DGAT1.a) was purified through initial solubilization in 1% n-dodecyl- $\beta$ -D-maltopyranoside (DDM), followed by cobalt affinity chromatography and size-exclusion chromatography (Caldo et al., 2015). The properties of BnaC.DGAT1.a in detergent micelles were characterized including the oligomerization states, specific activities at each stage of purification and substrate specificities. The first 113 residues of BnaC.DGAT1.a corresponding to a soluble regulatory region was expressed in *Escherichia coli* and purified. The role of this domain in oligomerization was investigated as well as its ligand binding properties.

**Results:** Purified BnaDGAT1 in DDM micelles predominantly exists as dimer, which can associate further to form tetramer. The major dimeric form was purified about 126-fold over the DDM-solubilized fraction and was found to prefer different acyl-CoAs in the following order:  $\alpha$ -linolenoyl-CoA > oleoyl-CoA > palmitoyl-CoA > linoleoyl-CoA > stearoyl-CoA. The N-terminal domain (BnaC.DGAT1.a1-113) was eluted from size-exclusion chromatography as a tetramer, which is in agreement with previous studies. Purification of truncated N-terminal domain revealed that residues 49-113 can associate to form dimer while the first 48 residues appear to be involved in tetramerization. When dissolved in a membrane-mimetic environment, BnaC.DGAT1.a1-113 assumes  $\alpha$ -helical structure as revealed by circular dichroism (CD). This N-terminal region was implicated as an allosteric exosite for acyl-CoAs as revealed by previous Lipidex-1000 binding studies. In the current study, ligand perturbation analysis monitored using CD also showed that oleoyl-CoA could interact with this regulatory domain. In addition, isothermal titration calorimetry showed that this interaction is an exothermic process and follows the sequential model for positive cooperativity.

**Conclusions:** DGAT1 appears to shift between two oligomerization states, a phenomenon that may be related to regulation of enzyme activity. This process appears to be mediated by the N-terminal region, which can bind acyl-CoAs through sequential positive cooperativity.

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## Optimized rapeseed oils rich in endogenous micronutrients ameliorate risk factors of *atherosclerosis* in high fat diet fed rats

**Background:** Micronutrients in rapeseed such as polyphenols, tocopherols, phytosterols and phospholipids in rapeseed exert potential benefit to *atherosclerosis* (Szydłowska-Czeraniak 2013). Some part of these healthy components substantially lost during the conventional refining processing. Thus some new processing technologies, such as cold pressing, dehulling-cold pressing and microwave pretreatment-cold pressing, have been developed to produce various endogenous micronutrient-enriched optimized rapeseed oils.

**Objectives:** The aim of this study is to determine the effects of the various endogenous micronutrient-enriched optimized rapeseed oils on atherosclerosis risk factors in rats fed a high-fat diet.

**Methods:** Rats received the high-fat diet containing 20% casein, 35% maize starch, 15% glucose, 5% cellulose, 3.5% mineral mixture (AIN-93M), 1% vitamin mixture (AIN-93M), 0.2% choline bitartrate, 0.3% DL-methionine and 20% fat. The refined rapeseed oil or optimized rapeseed oils obtained with various processing technologies as lipid source. After 10 weeks of treatment, plasma was assayed for oxidative stress, lipid profiles and inflammation.

**Results:** Micronutrients enhancement in optimized rapeseed oils significantly reduced plasma oxidative stress, as evaluated by the significant elevation in the activities of CAT and GPx as well as the level of GSH, and the significant decline in lipid peroxidation. Optimized rapeseed oil with the highest micronutrient contents obtained by microwave pretreatment-cold pressing reduced the levels of TG, TC and LDL-C as well as IL-6 and CRP in plasma.

**Conclusion:** These results suggested that the optimized rapeseed oils rich in endogenous micronutrients might contribute to prevent atherogenesis and make them very promising functional food in cardiovascular health promotion.

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## Determination for aflatoxins B1 in rapeseed oil using a rapid time-resolved fluorescent test strip

**Background:** Rapeseed oil is one of the most extensive consumed oils in China, covering about 50% of plant oil. Aflatoxin B1 (AFB1), produced by fungi, can seriously contain rapeseed oil throughout the whole production process, including before and after harvest and storage, transportation, and consumption. AFB1 has been proved to be toxic, mutagenic, teratogenic, and carcinogenic and can cause hepatic and extrahepatic carcinogenesis of humans and livestock. Thus, it is urgent to develop rapid determination method for AFB1 determination in rapeseed oils. Although numbers of studies have focused on the AFB1 determination in rapeseed oil, such as HPLC, LC MS/MS or ELISA, etc., these methods were hampered in practice due to the requests of specific instruments, skilled operators, laboring sample preparation, or the fake positive results. It is important to develop a rapid, sensitive, on-site determination for rapeseed oils. In this abstract, a test strip-based rapid, sensitive, on-site determination for AFB1 in rapeseed oil was developed and validated.

**Objectives:** It is to establish a rapid, sensitive, on-site determination for AFB1 in rapeseed oil, along with simplification of sample preparation. This method can afford a promising alternative for rapid, sensitive and on-site determination of AFB1 in rapeseed oil. This proposal can be used in the determination of AFB1 in rapeseed oil throughout the whole production. It also can provide key technique support in the government monitoring of the food safety in rapeseed oil.

**Methods:** The monoclonal antibody against AFB1 was domestically produced using the hybridoma antibody technology [1]. The covalent coupling of Eu<sup>3+</sup>-microbead and anti-AFB1 mAb or IgG were followed a modified EDC conjugation method. [2] The time-resolved fluorescent test strip was made by using the XYZ3050 dispensing Platform, CM4000 Guillotine Cutter, and LM4000 Batch Laminator (BioDot, Irvine, CA, USA). The immunoreagent concentrations on the test line and control line, along with the reaction conditions were optimized, respectively. This test strips were further evaluated, including limit of detection, linear range, recovery, precision, specificity, and agreement between the results of the test strip and HPLC method. Real rapeseed oil samples were finally tested using this proposed test strip.

**Results:** The results of Eu-based time resolved fluorescent test strip was recorded by using a portable reader within 10 min. Results found a limit of detection of 0.10 ng mL<sup>-1</sup>, a considerable linear range of 0.10-10.0 ng mL<sup>-1</sup>, recoveries from 85.10% to 115.25%. It was also found of excellent of precision (RSD: 6.8%) and specificity (RSD below 9.7%). Little cross-reactivity was found with AFB2, AFG1, AFG2, and AFM1). Excellent agreement of results between the test strip and HPLC methods was recorded, while 50 rapeseed oil samples from local markets were determined with the test strip, finding a AFB1 content of 0.35-4.26.

**Conclusions:** This test strip can be wide applied in the rapid on-site determination for AFB1 in rapeseed oils. This method allowed the rapidness, high sensibility, and low cost. It can be wide employed in the determination of AFB1 in rapeseed oil and further used in food safety monitoring.

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# Identification and quantification of glucosinolates in *Camelina sativa* seed by ultra performance liquid chromatography-photodiode array detector-tandem mass spectrometry

**Background:** *Camelina sativa* L. Crantz belongs to *Brassicaceae* family. It is heat and drought tolerant and is thought to require fewer agronomic inputs and is therefore a good alternative crop for marginal lands. Its seed contains three glucosinolates: glucoarabin (9-(methylsulfinyl) nonylglucosinolate or GS9), glucocamelinin (10-(methylsulfinyl)decylglucosinolate or GS10) and 11-(methylsulfinyl)undecylglucosinolate or GS11). The structures of these glucosinolates are similar to that of glucoraphanin (4-(methylsulfinyl)-butylglucosinolate). The degradation product of glucoraphanin is thought to be an anti-cancer compound.

High performance liquid chromatography (HPLC) is widely used to determine content of intact glucosinolates and desulfoglucosinolates. However, a typical HPLC gradient run time takes 25 to 50 minutes for intact glucosinolates and desulfoglucosinolates, hindering the camelina line screening process.

To better utilize camelina, we developed an Ultra Performance Liquid Chromatography-Photodiode Array Detector-Tandem Mass Spectrometry (UPLC-PDA-TQD) method for the analysis of desulfoglucosinolates with a reduced LC run time and solvent consumption. Then we surveyed six accessions for their glucosinolate content.

**Objectives:** Determine if UPLC can shorten the LC run time for glucosinolate analysis. Screen several Canadian camelina accessions to determine their glucosinolate profile and content.

**Methods:** Glucosinolates were extracted and converted to desulfoglucosinolates based on AOCs Official Method Ak 1-92. The analyses employed a Waters UPLC-PDA-TQD system equipped with a BEH Shield RP18 column (2.1 x 50 mm; 1.7  $\mu$ m) and held the initial conditions (100% water at 0.8 mL/min) for 0.3 minutes before a linear solvent gradient of 0% to 25% acetonitrile (v/v) over the next 6.7 min. The desulfoglucosinolates were quantified at 229 nm and identified by monitoring the characteristic loss of 162.2 mass units using MS/MS constant neutral loss scans.

**Results:** UPLC reduced the typical LC run time of 25-50 minutes for camelina desulfoglucosinolates to less than 10 minutes and consumed 60% less acetonitrile. Thus, it is possible to analyze more than 140 samples in a 24 hour period. When a typical HPLC separation is used, only 30-60 samples can be analyzed a day. The camelina accessions surveyed contained 21 to 31  $\mu$ mol glucosinolates per gram of seed. GS9 (4.6-7.0  $\mu$ mol/g seeds), GS10 (13.7-20.2  $\mu$ mol/g seeds) and GS11 (2.5-3.6  $\mu$ mol/g seeds), were identified and quantified. Among them, GS10 was the major glucosinolate. Additionally, we identified a putative minor glucosinolate, 8-(methylsulfinyl)octyl glucosinolate (GS8), in all six accessions based on its mass spectrum. GS8 content ranged from 0.06 to 0.13  $\mu$ mol per gram seeds. While GS8 is known in other plant tissues, this is the first report of GS8 in camelina seeds based on our knowledge.

**Conclusions:** UPLC reduced typical LC run time from 25-50 minutes to less than 10 minutes, greatly increasing our screening throughput and ability to detect minor glucosinolates in camelina and other crucifer species. The six camelina accessions surveyed contain glucosinolates at 20-30  $\mu$ mol/g seeds, with GS10 being the major glucosinolate in all accessions. A putative minor glucosinolate (8-(methylsulfinyl)octyl glucosinolate) was identified in these accessions. Further work is needed to confirm the identity of the minor glucosinolate.