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ORAL PRESENTATION THEME C

Structural organization of lipid droplets in two rapeseed genotypes is linked with seed lipid content and oil extractibility

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Background: The worldwide oilseed production will face an increasing demand in the next decades due to a higher consumption for edible oil, the development of the biofuel industry, and the needs of molecules for green chemistry. Seed lipids due to their diversity constitute a unique renewable source for food, feed, energy and chemistry. They are stored in specialized organelles, lipid droplets (LDs), with specific structure conserved among living organisms, a neutral lipid core surrounded by a monolayer of phospholipids in which a variable number of proteins is embedded (Huang 1992). Longly considered as inert balls of fat, LDs are now considered as organelles, with complex protein and lipid compositions and their own dynamics (Beckman 2006). In a former work, we have suggested a link between seed oil extractability and LD stability (Jolivet et al 2013). Deciphering the molecular basis of such link could help to save energy used during oil rapeseed extraction.

Objectives: LD stability is a consequence of protein – phospholipid interactions, and is partly responsible for the difficulty to extract oil from rapeseed. The respective role of these two components was evaluated using two rapeseed genotypes to perform a detailed study of LD characteristics in mature seeds as well as throughout seed development.

Methods: Amber and Warzanwski accessions were chosen because their mature seeds differ (i) in crushing ability evaluated by a micro-pressing technique (Savoire et al 2010), (ii) in oil extraction yield and, (iii) in the stability of their purified LDs. LD morphology and size determination were investigated by microscopy, and pulsed-field gradient NMR (PFG-NMR). LD composition into triglycerides, phospholipids and proteins was compared between the two accessions.

Results: PFG-NMR and microscopy allowed revealing in situ a LD size difference between Amber and Warzanwski. Amber LDs were enriched with H-oleosins and steroleosins suggesting a better coverage of LD surface. Their phospholipid composition showed an increase in phosphatidylserine content facilitating lipid-protein interactions and a decrease of poly-unsaturated species suggesting a more rigid structure.

Conclusions: PFG-NMR is a powerful non destructive method to characterize LDs in mature seeds. Differences found in composition of LD surfaces could explain uneven behaviours in Amber and Warzanwski seeds' treatment.

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ORAL PRESENTATION THEME C

HPLC determination of bioactive compounds in canola oil for varietal screening

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Background: Oil yield and oil stability in canola have been increased via successful breeding programs, but with the continuing growth of worldwide canola production, there is a need to improve the marketability of canola oil. Recent interest in the enhancement of potentially health-beneficial bioactive components present in the oil has developed (Ghazani et al., 2013; SzydłOwska-Czerniak, 2011). Tocopherols, sterols and carotenoids are classes of bioactive components present in crude canola oil that can exhibit health-benefits when incorporated into the human diet. Methods to quantify these compounds in food matrices often involve rigorous sample preparation that minimises analyte degradation, resulting in time and cost expenditure (Azmir et al., 2013).

Objectives: To develop a robust HPLC method to simultaneously analyse canola oil samples for tocopherols, carotenoids, free sterols and esterified sterols, which reduces sample preparation and analysis time in comparison to previous techniques.

To use this method to analyse a large population of Australian genotypes to determine genotype influence on the compounds of interest.

Methodology: A new method was developed using HPLC-MS/MS to simultaneously quantify tocopherols, carotenoids and sterols in both free and esterified forms in canola oil. DAD was used to monitor target wavelengths and provide a second level of quantification for some compounds.

This method forms the foundation for a study to investigate the influence of canola variety on these trace analytes using 64 different canola genotypes grown in controlled trials in two regions in Australia. REML analysis of G x E effects will be performed to illustrate the degree of influence, providing useful information for breeding programs targeting genotypes with enhanced levels of these compounds.

Results: The newly developed method reduced sample preparation time, reducing the overall time and cost associated with analysis. The high selectivity of the method allowed the individual quantification of co-eluting sterol compounds that has prevented the use of normal phase chromatography in the past. Further application of this method could result in the calibration of a rapid NIR method for use in industry.

Conclusions: The developed method will be applied in further studies examining genotype influence and processing and storage parameters aided to enhance the bioactive components in oil.

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ORAL PRESENTATION THEME C

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Reduction of sinapine content in rapeseed *(Brassica napus L.)* by induced mutations in sinapine biosynthesis genes

Background: Sinapine is the most prominent antinutritive compound in the seeds of oilseed rape *(Brassica napus L)*. A reduction in sinapine content could improve the quality of rapeseed meal as an animal feed and in food industry. As natural variation for seed sinapine content is limited in rapeseed, mutation screening is a reasonable approach to isolate low-sinapine genotypes.

Objectives: Our aim is to identify low-sinapine genotypes with loss-of function mutations in sinapine synthesis genes. We try to study gene dosage and background effects in mutant combinations.

Methods: We screened a winter rapeseed EMS TILLING population for sinapine synthesis mutants. Single mutants were combined by crossing and phenotyped using HPLC and enzymatic analysis.

Results: In *Brassica napus* seeds, two paralogs of the sinapine synthesis genes SGT and REF1 are expressed. We identified and combined two stop codon mutants of the SGT and the REF1 paralogs and a stop and a splice site mutant of the REF1 paralogs by crossing and analyzed the segregating F2 offspring. Sinapine contents in the double mutants dropped dramatically by up to 71%. F3 seeds with two stop codon mutations in REF1 genes had the lowest sinapine contents (2.4 mg/g) as compared to the EMS control (7.5 mg/g). A REF1 splice site mutation did not result in a decrease in seed sinapine content probably due to incomplete splicing. Significant depletion of SGT enzyme activity in developing seeds proved loss-of-function of both gene copies and ruled out background effects. REF1 enzyme activities showed minor reductions and pointed at different substrate specificities of the paralogs and the presence of unspecific aldehyde dehydrogenases.

Conclusions: We demonstrate that only the combination of different knock-down mutations drastically alters the composition of a major secondary metabolite. The results cast new light on the activities of gene paralogs in a polyploid species.

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ORAL PRESENTATION THEME C

Fractionation of rapeseed from oil extraction to minor products

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Starting with the composition of the seed, the presentation illustrates the breakdown of the seeds, mainly by mechanical processes.

The main product is still the oil. But since the demand for digestible vegetable proteins is increasing and different factors have a negative impact on the oil price, too, other by-products and new processes are becoming more interesting for oil producers and oil refining costs are more and more in focus.

Different steps will be illustrated: 1) Seed and pre-treatment, 2) De-oiling, 3) Oil and derivative processing, 4) Cake and flakes processing and finally there are some reflections on the By-products from both sides: 5) those from the oil fraction and those from the cake or expeller side.

The oil and the protein content, the hull thickness and therefore the fibre content, the sinapin and phytic acid content, the glycosinolate content etc. are not only given and fixed in the incoming raw material. All this can be influenced already with the first step: the pre-treatment and the kind of process used for the oil extraction.

Consequently, the oil refining starts between 10 ppm phosphorous or < 1000 ppm phosphorus in the oil depending on the extraction technique, but what is not in the extracted oil remains in the cake or expeller, meaning phosphorus, too. That influences the percentage of the substances in each fraction and ultimately the processes itself.

Sometimes the focus of the process is in the reduction of hulls and fibers in the cake used for feed without any reduction of the nativity of the proteins. Other research teams are looking for purified protein isolates with a high potential to adsorb water and/or oil or to be a good emulsifier. Research projects like SynRG (financed by FNR/BMELV) are supported in order to isolate minor components like polyphenols and to implement these in polymerization of dicarbonic acids made out of plant seed oils.

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ORAL PRESENTATION THEME C

Comparison of canolol content and antioxidant capacity of solvent-extracted oil from pretreated rapeseed with microwave

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Background: To overcome the low oil extraction yield problem in cold pressing, it is advisable to seek new pretreatment instead of the usual thermal treatment of seeds before pressing. By using microwave radiation, a higher extraction yield can be obtained because the cell membrane is ruptured (Azadmard-Damirchi et al., 2010; Uquiche, Jeréz, & Ortíz, 2008; Yang et al., 2013). Although oil extraction yield by pressing from oilseed increased according to the application of microwave radiation, a part of oil still remained in the pressed-cake and they can be extracted afterwards by solvent. When pressing the oil from rapeseed, some of the phenolic compounds are transferred to the oil and most of them remain in the meal. However, there is a lack of information in the literature on the effect of pretreatment with microwave of rapeseed on total phenolic and canolol contents in oil from rapeseed pressed-cake extracted by solvent . Moreover, there is a lack of information in the literature on the comparison of total phenolic and canolol contents, and antioxidant capacity between pressed oils from rapeseed pretreated with microwave and solvent-extracted oils from pressed-cake, too

Objectives: In this work, Rapeseed is pretreated with microwave, then, the seed is pressed and the press-cake is extracted with solvent. The effect of conditioning time on the amount of total phenolic and canolol content of the obtained oil during the microwave pretreatment of rapeseed is studied. Also, 2, 2-diphenyl-1- picrylhydrazyl (DPPH) and the ferric-reducing antioxidant power (FRAP) of both oil samples are monitored and the correlations are analysis between antioxidant capacities and phenolic content.

Methods: Rapeseed was treated with microwave under 800 W for 0, 1, 2, 3, 4, 5, 6, and 7 min at a frequency of 2,450 MHz, then, the seed was pressed and the press-cake was solvent extracted, and the influence of microwave pretreatment and extraction method on the content of total phenolic and canolol content, and antioxidant capacity of the pressed and solvent-extracted oil were evaluated. The influence of microwave pretreatment and extraction method on the content of total phenolic and canolol content, and antioxidant capacity of the pressed and solvent-extracted oil were evaluated. Canolol was synthesized according to the method described by Harbaum-Piayda et al. (2010). The identification of Canolol was carried out on the basis of nuclear magnetic resonance (NMR) and mass spectrometry (MS). The MS used for analysis was a hybrid, triple quadrupole/ linear ion trap mass spectrometer. The canolol content was quantified on the basis of the calibration curves using a chromatogram at 280 nm.

Results: The results indicated that the amounts of total phenolic and canolol present in the oil significantly increased and that their concentrations positively linear correlated with microwave time ($r = 0.918$ and 0.921) ($p < 0.001$). The contents of total phenolic and canolol of oil varied significantly ($p < 0.01$) depending on the extraction method besides microwave time, and total phenolic and canolol concentrations of the solvent-extracted oil were 13.20-32.28 mg/100 g and 29.66-163.85 µg/g. Also, the antioxidant capacities data obtained by the DPPH and FRAP procedures of oil significantly increased with microwave time (ffor the solvent-extracted oil, $r = 0.928$ and 0.959) ($p < 0.001$), and the DPPH and FRAP values of the solvent-extracted oil were 40.32-118.69 µmol TE/100 g and 232.70-445.14 µmol TE/100 g.

Conclusions: Microwave pretreatment of rapeseed benefited increasing the phenolic compounds and improving the antioxidant capacity of oil. Compared with the pressed oil, the solvent-extracted oil had the higher total phenolic and canolol content, and the antioxidant capacities determined by the DPPH and FRAP methods. However, the solvent-extracted oil had the higher acid value, peroxide value, lovibond color, and phospholipids content, and must be refined by degumming, deacidification, and bleaching.

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ORAL PRESENTATION THEME C

Bioactive compounds in canola meal

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Background: The meal which remains after canola oil extraction is of relatively low value and is used mainly for animal feed (Alashi et al., 2014). This meal may have additional value in the pharmaceutical industry if potential health beneficial bioactive compounds with the ability to combat several modern day ailments could be identified.

Objectives: Canola meal extracts should be prepared using different solvents and characterized. Identification and characterization of protease inhibitors will be undertaken. Invitro antioxidant and bioactive properties of extracts were determined and activities would be further investigated through cellular assays.

Methods: All canola meal extracts (CMEs) were named according to solvent used for extraction. The antioxidant activities in all these extracts were determined by reagent based assay along with High pressure liquid chromatography (HPLC) and Liquid chromatography–mass spectrometry (LCMS) (Obied et al., 2013). Chromatography was used to purify protease inhibitors. All extracts were used in the anticancer assay based on topoisomerase inhibition, antidiabtic activity by dipeptidyl peptidase IV (DPP-IV) enzyme inhibition, antihypertensive activity by angiotensin converting enzyme (ACE) inhibition. Antilipase and cellular assay was used to determine potential antiobesity properties.

Results: The acetone and methanol extracts showed higher antioxidant. The extracts showed varying levels of both the topoisomerase-1 poisoning and inhibition activities which are indicators of anticancer properties. Acetone, butanol, and hexane extracts showed antiobesity activity, inhibiting adipocyte differentiation without causing cell toxicity. Butanol, acetone and water extracts showed high antidiabetic activity by inhibiting the enzyme DPP-IV.

Protease inhibitors (PIs) were also extracted from canola meal and purified to homogeneity from two different canola genotypes. Canola genotype-1 showed very strong antidiabetic activity compared with genotype-2. Water extracts the purified protease inhibitor from genotype-1 showed strong antihypertensive activity through the inhibition of angiotensin converting enzyme (ACE).

Conclusions: These potential bioactive and health-functional properties of canola meal extracts may increase the profitability for farmers, processors, food manufacturers, and the pharmaceutical industry.

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ORAL PRESENTATION THEME C

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Digestibility of protein and energy increase with increasing protein content in rapeseed

Background: Rapeseed is hitherto mainly produced because of its valuable oil content, the residual meal after oil extraction is mostly considered as a medium valued protein product. The amino acid composition of rapeseed is well balanced, but the digestibility of protein and energy is low compared with soybean meal. This is mainly caused by the high content of cell wall materials - non-starch polysaccharides (NSP) and lignin present in rapeseed. The hulls constitute about 28-30% of the dry matter (DM) in oil-free rapeseed meal and contain the highest proportions of NSP and lignin. Thus, removal of hulls results in an improved digestibility of the protein, but to date no commercial beneficial dehulling process has been developed (Jensen et al. 1995). The increasing demand for protein to feed the worlds growing population may put more focus on rapeseed protein.

Objectives: The objective of this study was to evaluate digestibility of protein and energy in defatted rapeseed meal from seed with varying protein content.

Methods: The rapeseed investigated comprised eight seed samples of double low spring rape *(Brassica napus L.)* obtained from Danisco Seed A/S, Holeby, Denmark and thirty seed samples of double low winter rape *(Brassica napus L.)* from DLF-Trifolium A/S, Store Heddinge, Denmark. The rapeseed samples were milled and defatted by diethyl ether, air dried and analysed for chemical composition by traditional methods.

The defatted rapeseed was autoclaved at 107°C for 20 min before diet formulation in order to inactivate myrosinase and improve palatability. Standard digestibility and N balance trials with rats were performed as previous described on each of the thirty-eight defatted seed samples. The diets were formulated with rapeseed meals as the sole source of dietary nitrogen (15 g N kg-1). The remaining ingredients in the diet were maize starch, vitamins and minerals.

Results and discussion: Protein content varied from 17-26% of DM on whole seed basis, while oil content varied from 39-52% of DM and there was a significant negative linear correlation between protein and oil content (Y(% oil) = - 0.60 x (% protein) + 59.6(% oil); R2 = 0.21; P < 0.001).

The digestibility experiments showed a positive correlation between protein content in the defatted meal and digestibility of protein and DM (P<0.001). Thus, 1 % increase in protein will increase digestibility of protein with 0.3% units and energy with 1% units.

Conclusions: The presented results shows that the nutritional value of rapeseed meal will increase with increasing protein content in the seeds. Thus from an nutritional point of view and in order to meet the growing demand for protein in the world more focus on protein content in future breeding programmes would be desirable.

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Standardized ileal digestibility of amino acids from two winter rapeseed products fed to broiler chickens

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Background: Rapeseed products, which are valuable high-protein feedstuffs for poultry, have been the focus of extensive research recently.

Objectives: The aim of this study was to compare the standardized ileal digestibility of amino acids (SIAAD) from two winter rapeseed products fed to broilers.

Methods: Rapeseed cake and expeller from the rapeseeds of the Belarusian variety "Lider" were tested in this experiment. The methodology used in our study followed that proposed by Lemme et al. (2004). The experiment comprised 24 group cages with 12 birds per cage (a total of 270 birds). Three-week-old Ross 308 male broilers were assigned to 3 treatments with 8 replicates each. The main components of the experimental diets were rapeseed cake (treatment 1) and rapeseed expeller (treatment 2). Non-specific (basal) endogenous amino acid loss after feeding a N-free diet was determined in a separate group (treatment 3) of broilers. The obtained values provided a basis for the standardization of ileal digestibility coefficients. Until the end of week 3, male Ross 308 broilers received ad libitum a starter diet (commercial feed mixtures in meal form). After 7-day experimental feeding, all birds were sacrificed by cervical dislocation and digesta was immediately sampled from the distal two-thirds of the intestine section between Meckel's diverticulum and 2 cm anterior to the ileo-ceca-colonic junction. The intestine contents were pooled (within each pen), freeze-dried and subsequently analyzed for amino acid content. The calculations were conducted using the formulas proposed by Lemme et al. (2004).

Results: Rapeseed cake contained 32.0, 1.9, 0.8, 1.3, 2.2, 1.8, 0.6, 1.3, 1.4, 0.4, 1.6% and rapeseed expeller contained 33.8, 2.1, 0.8, 1.3, 2.3, 1.8, 0.7, 1.4, 1.5, 0.5, 1.7% of CP, Arg, Cys, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, respectively. The total glucosinolate content of rapeseed cake and expeller was 31.94 and 19.13 µmol/g, respectively. The following standardized amino acid digestibility coefficients were determined: rapeseed cake - 88.0, 73.5, 78.8, 83.4, 77.1, 87.9, 80.4, 77.7, 71.3, 74.1 and 76.9%, rapeseed expeller - 92.0, 83.4, 84.6, 88.3, 85.3, 92.0, 87.4, 85.3, 79.9, 81.1 and 83.3% for Arg, Cys, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Trp and Val, respectively.

Conclusions: Both rapeseed products were characterized by good AA digestibility, however the SIAAD coefficients of rapeseed expeller were higher than those obtained for rapeseed cake. The values from the present study are close to or slightly higher than those reported by Lemme et al. (2004) and Szczurek (2006) for broiler chickens fed diets containing rapeseed cake.

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ORAL PRESENTATION THEME C

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Importance of linoleic acid in α-linolenic acid conversion to LC n-3 PUFA during fat substitution

Background: Arachidonic (AA, 20:4n-6), eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids, synthesized by the liver from dietary linoleic (LA, 18:2n-6) and α-linolenic (ALA, 18:3n-3) acids, are predominant long-chain polyunsaturated fatty acids (LC PUFA) in plasma phospholipids (PL) (Rapoport 2013). Furthermore, the conversion of ALA to DHA may occur selectively in the brain. (Barcelo-Cobljin and Murphy 2009). In our rapeseed oil (RSO) studies (Seppänen-Laakso et al. 2002, 2010) ALA exhibited significant competitive effects by increasing LC n-3 PUFA and inhibiting LA conversion to AA.

Objectives: Individual fatty acid data (baseline, 3 and 6 weeks' values) of the subjects (n=148) were taken from our previous studies, and the effects of plasma PL saturated fat and LA on LC PUFA were examined during fat substitution (nine groups including controls).

Methods: The groups changed butter to 1a) RSO (n=20, LA 24%, ALA 10%) or 1b) test margarine (n=23, LA 28%, ALA 3% with 18:1tr 16%), and margarine (LA 33%, ALA 2% with18:1tr 8%) to RSO (2a, n=23) or olive oil (2b, n=23, LA 9%, ALA 1%). Two groups (3a, n=32) and a third group (3b, n=10) received also RSO, with daily dose of 16 ml (3.4 g LA and 1.8 g ALA). Further, a small group received soybean oil (4, n=6, LA 55%, ALA 7%).

Results: In RSO group 1a, 20% of the increases at 3-6 weeks, showed the highest DHA response for DHA-AA-EPA combination (average DHA rise 2%-units, range 1.6-3%-units), while lower response (1%-unit) was typical in AA-DHA profiles (28%) without EPA. Low DHA (±1%-unit) in group 2a was combined with EPA (28%), whereas in groups 3a-b increases in AA-DHA profile (24%) with no EPA were characteristic. In groups 1b, 2b and 4, unexpected rapid decreases in LC n-3 PUFA at 6 weeks (60%) were found.

Conclusions: The profiles well reflect the balancing steps between major LC PUFAs AA, DHA and EPA. Not only linoleic acid but also saturated fatty acids strongly affect the conversion of ALA to LC n-3 PUFA. Lack of EPA in AA-DHA combination, in turn, would suggest specific conversion for ALA.

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ORAL PRESENTATION THEME C

Genetic classification and diversity of yellow-seeded rapeseed *(Brassica napus L.)* accessions

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Background: Yellow-seeded oilseed rape *(B. napus)* has thinner seed hull, less fiber, higher oil and protein content than its black-seeded counterpart so yellow seeded trait is valuable in oilseed rape breeding.

Objectives: We collected and developed various yellow-seeded accessions varying from pale to lemon, dotted yellow, greenish, reddish and yellowish brown colors in seed coat. In order to use these accessions in conventional and hybrid breeding, we selected some representative yellow-seeded accessions to perform genetic analysis.

Method: We investigated the allelism of seed coat color genes using the F1 and F2 populations of different crosses and studied genetic diversity using 48 SSR markers evenly distributed in A and C genome.

Results: Eleven yellow-seeded rapeseed accessions varying in seed coat colors from dotted yellow, greenish brown, reddish brown to yellowish brown were divided into five groups: group I containing Youyan10, CZV55, E718, and Armand-ys showing dominant inheritance in most crosses; group II including reddish brown Q33 and D615 showing incomplete dominance; group III consisting of 2006C, X2006, and 740C showing yellow seed coat as recessive trait in most cases; group IV having only yellowish brown Polo-ys and group V, one accession HY15. Most accessions in the last three groups showed a recessive trait of the seed coat colors while dominance or recessiveness of the seed coat colors in group I such as Youyan10 and group III such as 2006C varied when these accessions were crossed with different brown-seeded accessions. When Chinese varieties Yangyou4, Zheyou50, and Za77 were crossed with the yellow-seeded accessions of group I, all F1 plants had black seeds. On the other hand, when the European variety Solida and some Canadian spring varieties were crossed with the accessions in group III, all F1 plants had yellow seeds. Phylogenetic analysis based on SSR molecular markers showed that yellow-seeded accessions could be divided into eight subgroups, each of which were represented by 2006C and 740C, Youyan 10 system, Q33, GQ4 from Shaanxi, Ramiro-ys from French, Armand-ys and Profit-ys selected from Canadian cultivars, and Polo-ys from Polish cultivar Polo. The phylogenetic tree generally agreed with the pedigrees and sources from breeders and originations.

Conclusion: The complex allelic and epistatic interactions among different yellow-seeded accessions could be attributed to genetic regulation networks and genome duplication in the evolution of allotetraploid *B. napus*. The phylogenetic analysis in our research provides some theoretical basis for the heterosis utilization of yellow- seeded oilseed rape.

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ORAL PRESENTATION THEME C

Monitor plan of rapeseed quality in China

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Background: China is the largest rapeseed production country in the world (Zhang, 2007). In the past 15 years, the rapeseed breeding scientists bred a series of double-low rapeseed varieties. The yield and quality of rapeseed significantly improved.

Objectives: The aim of this study was to describe the change trend of quality of varieties and commodity rapeseed of double-low rapeseed during the past 15 years in China.

Methods: According to stratified random sampling method, more than 1000 samples of varieties and commodity rapeseed were collected from the main producing area every year. The oil content, glucosinolate and erucic acid were analyzed according to the National or International standard methods. The SAS and SPSS statistical tools were used to evaluate the changes of quality of these double-low rapeseed samples in China.

Results: In the past 15 years, the average oil content of rapeseed steadily increases and achieves exceeding 4% increase. The erucic acid contents of varieties and commodity rapeseed were 0.17% and 6.57%, which decrease by 94% and 60% respectively. The erucic acid contents of the new varieties approached to the international level. Meanwhile, the glucosinolate content of varieties and commodity rapeseed were 24.42mmol/g and 36.49mmol/g, which decrease by 31% and 44%, respectively. The rate of double-low rapeseed varieties and commodity rapeseed grew significantly, reaching to 99.53% and 71.19%, respectively.

Conclusions: In this study, the qualities and change trend of varieties and commodity rapeseed in China were summarized and analyzed. The qualities of both varieties and commodity rapeseed significantly increase in the past 15 years. Some of quality parameter of new varieties reaches to the international level.

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Domestication and molecular mechanism underlying yellow seed in *Brassica juncea (L.)* Czern & Coss

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Background: Seed color is not only a morphological indicator, but also a major agronomical character associated with seed quality and seed dormancy. In *Brassica* species, yellow seed has thinner testa, less fiber, and more oil and protein than their black- or brown-seeded counterpart. Seed color is controlled by proanthocyanidins (PA) deposited in its testa. In *Brassica juncea*, seed color is controlled by duplicate loci mapped on the chromosomes A09 and B03.

Objectives: First, what genes do these loci encode in *B. juncea*? Then, How do these genes regulate seed color at the molecular level? Furthermore, how many alleles do these genes each have in *B. juncea*? When, where and How is *B. juncea* yellow seed domesticated? It is of great significance in science and breeding to elucidate these questions.

Methods: The segregating backcross populations were used for positional cloning of the genes for seed color. The yeast one hybrid (Y1H), RNA-seq, and restriction digest of amplified fragments for expression analysis were used to uncover the molecular mechanism underlying seed color. Allelic variation was analyzed in a collection of *B. juncea* accessions from over thirty countries. Genetic analysis and historical literature records were used to elucidate domestication of yellow seed.

Results: The cloned seed color genes mapped on the chromosome A09 or B03 both encode a bHLH transcription factor orthologous to *AtTT8* in *Arabidopsis thaliana*. The yellow-seeded parent Sichuan Yellow (S) has a 1275 bp inserted fragment between nt3047-nt3048 and a substituted base at nt 3046 of *BjuA09.TT8* and at nt2742 of *BjuB03.TT8* compared to the black-seeded parent Ziyejie (Z). The mutated Bju.TT8 genes were expressed in testa of S and its brown-seeded near-isogenic lines. However, TT8-regulated genes such as dihydroflavonol-4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX) and anthocyanidin reductase(ANR) for PA biosynthesis, were not expressed in S testa. The mutated *Bju.TT8* genes from S did not interacted in vitro with the promoters of both DFR genes from *B. juncea*. Analysis of variation in *Bju.TT8* of about 200 *B. juncea* accessions found three additional loss-of-function *BjuA09.TT8* alleles including two insertions and one deletion. The mutated *BjuA09.TT8* alleles occur in all subspecies of *B. juncea* although the mutated *BjuB03.TT8* allele occurs only in yellow-seeded mustard.

Conclusions: Concurrent mutations in both *BjuA09.TT8* and *BjuB03.TT8* genes are responsible for spontaneous yellow seed in *Brassica juncea*. The yellow seed is domesticated by artificial selection, most probably in China, after speciation of *B. juncea*. The mutated *Bju.TT8* genes, although transcribed, can not activate expression of downstream genes so that yellow seed lacks deposition of PA in its testa.

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ORAL PRESENTATION THEME C

Develop an extrusion-assisted extraction process to increase canola and industrial oilseed meal inclusion in feed

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Background: Canola and rapeseed meal have been used as viable alternatives to soybean meal in feeds. However, there are anti-nutritional factors, such as glucosinolates and a higher content of fiber in meals, restricting their application at high inclusion levels in monogastric animals and poultry diets (Zhou et al. 2013). Additionally, lowering glucosinolates content in non-"double zero" oilseeds meal to levels typically found in canola meal would provide opportunities for these meals to be used in feeds, which makes biodiesel processors to be more competitive (Marillia et al. 2014). Moreover, glucosinolates is a natural preservative potentially used for pest control, and possible drug ingredients.

Objectives: The objective of this research was to develop an extrusion-assisted extraction process to (a) reduce glucosinolates content in canola and other meal; (b) increase digestibility of fibre; and (c) extract and recover glucosinolates as a natural preservative. This novel process is to replace the batch extraction process, enabling to carry out thermomechanical and chemical treatments in a continuous step.

Methods: A 40-mm twin-screw extruder (Century Extrusion, Traverse City, MI, US) was employed. Experiments were performed according to a factorial design with solvent, processing profile such as screw speed, solvent / solid ratio as the factors. The level of glucosinolates, crude fibre, and neutral detergent fibre in extrudates were measured, and compared with those in raw canola meal. The optimized process was selected by the most efficient combination of factors.

Results: The level of glucosinolates in extruded meal is lowered, and the fibre components in extrudates have been modified with a positive potential on the intake by animal. Extrusion assisted extraction process was validated.

Conclusions: The optimized process depicted the main advantages of this process such as reduced time, solvent, and reactant requirements; enhanced extraction yield and rheological properties; and high purity of the product. Further research is needed in terms of isolation of glucosinolates in liquid stream, and animal test.

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Volatile aroma compounds as markers for the assessment of the sensory quality of virgin cold-pressed rapeseed oil

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Background: Virgin cold-pressed rapeseed oil becomes more and more attractive to the consumer as an alternative or in addition to virgin olive oil. The advantages of this type of oils compared to common refined edible oils are less processing, natural content of nutritionally important compounds, the intensive colour and the typical seed-like and nutty taste and smell. Although the process itself is simple with pressing of the rapeseed by a screw press and purification of the oil by filtration, sedimentation or centrifugation, the processing of high-quality virgin cold-pressed rapeseed oil is an art. Most important is the choice of the raw material that directly influences the quality of the oil. One important tool for the quality control of virgin cold-pressed rapeseed oil is the sensory assessment which is performed by at least 3 to 5 trained persons under standardized conditions. For rapeseed oil the method DGF-CII 1 (14), Appearance - Sensory assessment, can be used as a reliable, but personnel and time-consuming method with some uncertainties. A promising approach to support the sensory assessment of virgin cold-pressed rapeseed oil by analytical means is the profiling and characterization of volatile compounds in combination with statistical methods.

Objectives: The aim of the present work is to find analytical methods for the classification of virgin cold-pressed rapeseed oil in sensory good and bad oils as well as to find volatile compounds that are responsible for the typical aroma and specific off-flavours of the rapeseed oils, respectively.

Methods: The volatile compounds are extracted by dynamic headspace (DynHS), determined by gas-chromatography (GS) with FID and identified with MS detection. Aroma active compounds are detected by GC-olfactometry (GC-O) and matched with corresponding GC-MS peaks, while the identification is done by comparison with analyzed standard substances and with help of NIST-databases. Relationships between compounds and differences between samples on basis of the volatile compounds are investigated by statistical means.

Results: The profiles of volatile compounds from a sensory good rapeseed oil and a corresponding rapeseed oil with sensory defects such as fusty and musty are measured by DynHS-GC-MS and the aroma active compounds were identified by DynHS-GC-O. Additionally a dataset of 43 samples has been classified into two groups of sensory good and bad oils according to the sensory assessment of a panel group as basis for an automated analysis by statistical means. 64 volatile compounds have been detected on basis of DynHS-GC-O/MS data of which 41 compounds were described as aroma-active substances. 23 of all detected volatile compounds show significant differences in the peak intensities between sensory good and bad oils. Especially carboxylic acid esters occur with higher intensities in the sensory bad quality oils. Altogether 46 volatiles could be identified. A dataset of 41 of these identified volatile compounds together with the use of the Principle Component Analysis (PCA) results in a partial differentiation of sensory good and bad oils.

Conclusions: DynHS-GC in combination with statistical means can be a helpful tool to support the sensory analysis and assessment of virgin rapeseed oils. Further development is necessary.

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ORAL PRESENTATION THEME C

Canolol enriched extract from heat-treated canola meal as an option to improve frying stability of high-oleic canola oil

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Background: In comparison to other oilseeds rapeseed contains relatively high amounts of phenolic compounds, mainly derivatives of sinapic acid but during oil processing only small parts of the phenolic compounds go into the oil while most of the compounds remain in the press cake. During heating of the raw material sinapic acid is degraded by decarboxylation into the oil-soluble 2,6-dimethoxy-4-vinylphenol (vinylsyringol or canolol) which is described as a strong antioxidant in different systems.

Objectives: In the present work the effect of a 2.5% canolol-enriched extract obtained from the extraction of fluidized bed treated rapeseed meal with super-critical carbon dioxide should be investigated in a deep-fat frying study.

Methods: The extract was added to high-oleic rapeseed oil in amounts corresponding to 200, 500 and 750 mg/kg canolol and the effect on the formation of di- and polymer triacylglycerols, total polar compounds, secondary degradation products (anisidine value) and the iodine value during deep-fat frying was compared to commonly used antioxidants TBHQ (200 mg/kg) and rosemary extract (40 and 200 mg/kg) as well as a control without antioxidant.

Results: The canolol enriched extract showed a three times stronger effect on stabilizing the frying oil during processing than TBHQ or rosemary extract. The extract was also able to reduce the degradation of α- and g-tocopherol during frying. The LD50-values for the degradation of tocopherols for the different amounts of added canolol enriched extracts ranged between 20.2 and 28.7 h for α-tocopherol and 15.8 and 19.6 h for g-tocopherol, while the LD50-values for the other antioxidants were between 3.6 and 7.7 h, and 4.5 and 8.3 h, respectively. The canolol enriched extract showed a strong concentration-dependent performance with a better effect with a higher concentration.

Conclusions: The experiment showed that the addition of canolol enriched extract can be a promising possibility to enhance the frying performance of vegetable oils. Fluidized bed treatment of rapeseed meal is an interesting opportunity to induce the formation of canolol making rapeseed meal to an interesting raw material for an antioxidant effective extract. This gives rapeseed meal an added value. Investigation on the composition of the extract as well as on antioxidant compounds besides canolol will be continued.

QTL analyses reveal pleiotropic effects of erucic acid and glucosinolate content on other seed quality traits in oilseed rape

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Background: Traditional oilseed rape contains up to 60% erucic acid in the seed oil and about 90 µmoles of glucosinolates in the seeds. Through the conversion to canola quality oilseed rape, contents of those two constituents have been reduced to almost nil. This must have caused remarkable changes in the biosynthesis of other primary and secondary seed quality components. However, the biochemical consequences of the conversion to canola quality are not at all yet clear.

Objectives: To study the effect of conversion from traditional oilseed rape to canola quality on the contents of other primary and secondary seed components.

Methods: A number of DH populations segregating for erucic acid and glucosinolate content were analysed for their contents of other primary and secondary seed components (Amar et al. 2008; Schatzki et al. 2014, Suprianto 2014)

Results: Doubled haploid populations segregating for erucic acid and glucosinolate contents have been studied in field experiments and a number of pleiotropic effects of those two constituents have been detected in QTL analyses. Erucic acid content did not only influence oil content but also contents of sinapate and phytosterols (Amar et al. 2008). Glucosinolate content influenced seed storage protein composition (Schatzki et al. 2014) and seed fibre composition (Suprianto 2014).

Conclusions: The pleiotropic effects of erucic acid and glucosinolate content on other seed quality traits limits the application of NIRS calibrations and other analytical methods which are routinely used to screen *Brassica* genetic resources for valuable seed quality traits. Care must be taken to avoid detecting spurious genetic variation in genetic resources which may be caused by cross-correlation to erucic acid and/or glucosinolate content. The relevance of those results for further genetic improvements of seed quality traits in oilseed rape will be discussed.

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ORAL PRESENTATION THEME C

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Cold pressed canola oil- applying terroir concepts to a aommodity crop

Background: Cold pressed canola oil processing is of increasing interest to canola growers as a value-added income stream. In partnership with the Manitoba Canola Growers Association, NuEats Food Innovation Inc has developed a scalable, low input pressing and clarification regime suitable for on-farm or small enterprise implementation.

Objective: To leverage increasing consumer and food service interest in the concept of 'terroir'- commonly acknowledged as the set of special characteristics that the geography, geology and climate of a region, interacting with germplasm, expresses in the final product.

Methods: Multiyear samples of the same cultivar of canola grown in three distinct geographic regions in Manitoba were sourced, cleaned, crushed and bottled. The resulting cold press oil was evaluated for fatty acid profile, nutrition, appearance, cooking performance and sensory characteristics. Test marketing results were used to identify key attributes and pricing.

Results: Cold pressed canola oil samples demonstrated differences in fatty acid profile and sensory characteristics compared to traditional processed canola oil.

Conclusions: Regionally sourced cold pressed canola oil offers a value added opportunity for growers and is well suited to on farm enterprise. Acceptance was high with consumer as well as food service users.

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ORAL PRESENTATION THEME C

Canola meal replacing wheat-DDGS as protein source for dairy cows

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Background: Traditionally, dairy cow diets in western Canada contain canola meal (CM) as the principal source of protein because it is a high quality protein supplement (Hickling, 2008). On the other hand, growth of the ethanol industry using wheat as a feedstock has resulted in large quantities of wheat dried distillers grains with solubles (W-DDGS) being available as an alternative protein supplement. When compared with CM, W-DDGS contains less CP (37 vs. 42%) and is a poorer source of lysine (2.5 vs. 5% of CP; Maxin et al., 2013); thus, feeding W-DDGS in place of CM could potentially compromise cow performance due to a lysine deficiency. Also, dietary CP content can have major effects on ruminally-degradable protein (RDP) supply as the amount of dietary CP that is degraded in the rumen increases with dietary CP content.

Objectives: To determine the effects of feeding CM or W-DDGS in diets varying in CP content on ruminal N utilization, omasal flows, and milk production.

Methods: Eight multiparous Holstein cows were used in a replicated 4 × 4 Latin square design with 28-d periods. Treatments were: 1) source of protein (CM vs. W-DDGS); and 2) dietary CP content (15 vs. 17%). Feed intake, and milk production were measured during the last 8 d of each period. Omasal digesta flow was quantified using indigestible NDF, YbCl3 and Cr-EDTA as digesta markers, whereas ruminal microbial protein production was quantified using (15NH4)2SO4 as a microbial marker.

Results: DM intake and milk yield were unaffected by diet; however, numerically, cows fed CM produced 1 kg/d more milk when compared to cows fed W-DDGS. DM apparently digested in the rumen was greater in cows fed the high CP compared to those fed the low CP diet, with the difference in DM apparently digested in the rumen being greater in cows fed W-DDGS as compared to those fed CM (interaction, P = 0.02). RDP supply was greater in cows fed the high CP when compared to those fed the low CP diet when diets contained CM, whereas RDP supply was lower in cows fed the high CP when compared to those fed the low CP diet when diets contained W-DDGS (tendency for interaction, P = 0.08). RUP supply was greater in cows fed the low CP when compared to those fed the high CP diet when diets contained CM, whereas RUP supply was lower in cows fed the low CP when compared to those fed the high CP diet when diets contained W-DDGS (tendency for interaction, P = 0.06). Omasal flows of threonine and tryptophan were greater (P ≤ 0.03), whereas that of histidine and lysine tended (P ≤ 0.08) to be greater, in cows fed CM when compared to those fed W-DDGS.

Conclusion: When diets are formulated to contain 15 or 17% CP, CM or W-DDGS can support similar levels of milk production.

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ORAL PRESENTATION THEME C

Molecular mapping and QTL for seed pigmentations and 19 genes of flavonoid pathway in *Brassica napus L.*

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Background: Expression quantitative trait loci (eQTL) can detect the expression of a specific gene and the genotype at that gene's locus, as well as evidence for clustered trans-eQTL that simultaneously regulates a large fraction of the transcriptome (Morley et al. 2004). In *B. napus*, the seed coat colour is determined by the phenolic compounds and procyanidins (Lepiniec et al. 2006; Qu et al. 2013). Dozens of genes involved in flavonoid biosynthesis pathway had been elucidated in Arabidopsis, and much more homologs had been found in *B. napus* (Chai et al. 2009; Chen et al. 2013). However, the function of these genes for *Brassica* yellow seed trait formation is still not well understood.

Objectives: This research focused on QTL identification responsible for four seed pigmentations and nineteen genes in flavonoid pathway to systematically elucidate the characteristics of flavonoid pathway, and provide the necessary information for seeking the key genes or regulation nodes controlled the yellow seed trait formation in *B. napus*.

Methods: We employed a sample of 94 recombinant inbred lines (RILs) from a population derived from a cross between black-seeded male parent cultivar Zhongyou 821 and yellow-seeded female parent line GH06. Major QTLs controlling four kinds of seed pigmentations were identified by genetic map construction. Then, transcript-level variation analysis was carried out on RNA from seeds of 30 days after flower (DAF) by qRT-PCR. Regarding as quantitative traits, the transcript levels of the flavonoid biosynthesis genes families were examined by QTL mapping method for eQTL detection. To confirm the candidate genes, sequences of association makers were used for BLASTN search in the BRAD and our local *B. napus* genome sequence database.

Results: A total of 57 QTLs for seed pigmentations and 75 eQTLs for nineteen genes were detected and distributed among 15 different linkage groups. Interestingly, 4 hotspot regions including 19 QTLs and 30 eQTLs were identified and distributed on the chromosome A03, A09 and C08, respectively. Besides, the most interesting hotspot in our study was the lower hotspot on chromosome A09, showed well synteny to genome sequences of *A. thaliana, A. lyrata* and *Brassica* relatives. A total of 8 transcription factors were identified in this region, three of them belongs to the flavonoid biosynthesis related MYB transcription factor family. Additionally, In trans-eQTL hotspot on chromosome A03, C08 and the upper A09, we identified 5, 1 and 10 transcription factors, respectively. Among these transcription factors, bZIP25, MYC1 and other function unclear transcription factors could be regarded as candidate genes in the trans-eQTL hotspots for further verification.

Conclusions: Molecular markers closely linked with these QTLs could be applied in marker-assisted selection of improving of feeding value of rapeseed. The functions of these candidate genes will provide insight into the molecular and biochemical mechanism of seed coat development in *Brassicaceae*, and elucidate the regulatory network underlying seed coat colour formation in *B. napus*.

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High inclusion levels of canola meal in laying hen diets

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Background: Canola meal (CM) inclusion levels in laying hen diets have traditionally been limited to a maximum level of 10% due to the presence of anti-nutritional factors, including glucosinolates, sinapine or tannins. The content of glucosinolates in canola meal has declined significantly over the years as a result of selection pressure by canola breeders. Based on the recent survey involving 11 Canadian crushing plants, the level of glucosinolates in CM averaged 3.9 µmol/g (Rogiewicz et al., 2012). Therefore, the rations for laying hens could now contain significantly more CM without causing any adverse effect on egg production or mortality due to hemorrhagic liver syndrome. In this context, Khajali and Slominski (2012) indicated that a dietary level of glucosinolates of 1.5 µmol/g would have no negative effect on laying hen performance

Objective: To investigate the effect of different dietary levels of CM on egg production and egg quality parameters in laying hens.

Methods: A wheat/corn/SBM-based Control diet, and diets containing 4, 8, 12, 16 or 20% of CM were fed to 6 replicate cage units of 18 Lohmann LSL laying hens each per treatment throughout the 24-week study consisting of 2 phases and three 28-d periods in each phase. Diets were formulated to contain 17.0 and 16.4% of CP and 2,800 and 2,700 kcal/kg of metabolizable energy in Phase 1 and 2, respectively. Dietary glucosinolate content averaged 0.28, 0.52, 0.97, 1.30, and 1.49 µmol/g for the diets containing 4, 8, 12, 16 or 20% of CM, respectively. Hen-day production, egg mass, feed intake, and feed efficiency were determined three times in each phase at the end of a 28-d period. All eggs were weighed for 3 consecutive days in the middle of each period and 36 eggs per treatment were selected for egg quality measurements, including albumen height, Haugh units, specific gravity, yolk color, and egg shell elasticity and thickness.

Results: There were no significant differences in hen-day production, feed intake, feed efficiency, and mortality between dietary treatments ($P>0.05$). Egg weight was slightly higher ($P<0.05$) in the control group than in hens consuming 16 and 20% of CM in phase 1 (64.4 vs. 63.3 and 63.3g, respectively) and phase 2 (63.4 vs. 62.4 and 62.2 g, respectively) of the experiment. However, the egg mass was not affected as a result of the same or better hen-day egg production in hens consuming CM-containing diets. When compared with the control diet, different dietary levels of CM had no effect on egg and egg shell quality parameters.

Conclusion: It would appear evident that CM could replace SBM and used effectively in laying hen diets at the dietary level of 15-20%.

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ORAL PRESENTATION THEME C

Prospects of water-lean protein recovery from rapeseed press cake

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Background: Production of protein-rich ingredients from rapeseed cold pressing residue has potential to bring additional value to the oil pressing industry. Commercialization of rapeseed protein production is, however, hindered by high investment and operational costs of the current water and energy-intensive processes. Rapeseed protein is generally extracted from the press cake with alkali or salt solution at 5-10% total solid content and recovered by acid precipitation. Although protein fractions with high purity can be obtained, introduction of salt into the protein fraction results in the need for additional washing steps. Instead of aiming at pure isolates, production of protein concentrates by simple, water-lean methods could improve the economics and sustainability of the concept without compromising technical properties of the product.

Objectives: The aim was to develop water-lean protein extraction and dry fractionation technologies for rapeseed press cake to improve technical and economic feasibility of rapeseed protein production.

Methods: Press cake from cold pressing of turnip rape *(B. rapa)* seeds was defatted by supercritical CO2 extraction and dry-milled. Two extraction processes were applied at increased total solid content to recover protein-rich fractions from the defatted press cake: 1) alkaline extraction followed by isoelectric precipitation and drying of the protein-rich precipitate, and 2) water extraction and drying of the protein-sugar extract. Carbohydrate-degrading enzymes were utilized to facilitate the recovery of protein extracts as described by Rommi et al. (2014). The effects of enzyme treatment and total solid content on the yield and production costs of protein-rich fractions were determined. As a water-free approach, protein content the defatted press cake was enriched by dry fractionation. The influence of particle size and operational factors on the separation of protein-rich kernel and fiber-rich hull particles by air classification were investigated.

Results: In order to extract over 50% of the protein from defatted rapeseed press cake at 20% solid content, alkaline pH or additional extraction rounds were needed. Carbohydrate-degrading enzymes disrupted the cell wall matrix, functioned well also at 40% solid content, and facilitated the recovery of protein extracts. Although enzyme-aided water extraction gave low protein yields at increased solid content, the resulting protein-sugar extracts possessed better dispersion stability than isoelectric protein precipitates from alkaline extraction. Raw material and energy formed the major costs of the evaluated extraction processes, and spray drying of the product and processing residues was indicated as the most energy-intensive phase. Air classification of defatted press cake produced light-colored, hull-free fractions with ca. 40-45% protein content.

Conclusions: Three water-lean concepts were developed to produce protein-rich fractions from rapeseed press cake. Due to significant drying costs in dilute processes, increasing the total solid content of extraction processes seems favorable despite reduced protein yield. Alkaline extraction and isoelectric precipitation is feasible at 20% solid content; however additional salt removal steps and technological functionality of the protein precipitates need to be considered. Enzyme-aided water extraction produces protein-sugar fractions with better dispersion stability, but the protein yield is compromised unless additional extraction rounds are used. Dry fractionation represents a promising alternative approach to be further developed to obtain fractions with higher protein content.

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ORAL PRESENTATION THEME C

Toasting and amino acid availability of rapeseed meal in pigs

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Background: In oilmills, heat and steam in the desolventizer/toaster (DT) evaporate the hexane from the de-oiled rapeseed cake (RSC), thereby producing solvent-free rapeseed meal (RSM). A side effect is the degradation of glucosinolates (GSL), and an excessive heat treatment may lower the content of amino acids (AA) and their availability expressed as standardized ileal digestibility (SID).

Objectives: Variation of toasting in oil mills was to simulate by processing one batch of a defined rapeseed (RS) into 5 RSM, varying in their residence time (RT) in the DT. These RSM should be analyzed for GSL and AA and in pigs studied for the SID of AA.

Methods: In the DT of CREOL pilot plant, from the RSC soaked with hexane 4 RSM were produced under wet toasting conditions (WetTC) with increasing RT of 48, 64, 76, and 93 min. A fifth RSM representing 70 min mean RT was 60 min additionally processed (RSM 70+60), mainly by dry toasting conditions (DryTC). Six barrows (initial BW = 22 ± 1 kg), fitted with a T-cannula at the distal ileum, were allotted to a 5 × 6 row column design with 5 RSM-casein-cornstarch diets and 5 periods. The SID of AA in the RSM was determined as difference to the SID of casein AA.

Results: Increasing the RT reduced the GSL content – in RSM 70+60 by 90% of the GSL in RS defatted matter. Also the contents of lysine (inclusive its reactive part in guanidination) and cystine were diminished. The SID of most AA in RSM decreased linearly ($P < 0.05$) as the RT in the DT increased from 48 to 93 min - till 10 and 11%-units decrease for lysine and cystine SID ($P < 0.05$). The additional DryTC (RSM 70+60) resulted in SID values similar to RSM93.

Conclusions: For pigs an improved acceptance of longer heated RSM with very low GSL content is compromised by decreased content of limiting AA such as lysine and also by a lowered SID of most AA. However, a loss of AA due to longer toasting could be compensated by AA supplements. Considering further RSM quality criteria as protein solubility or fiber-fixed N, the toasting can be optimized. In conclusion, under the frequently used WetTC, the RT should not exceed 60 to 75 min.

Acknowledgement: This study was supported by the Union for Promotion of Oil and Protein Plants (UFOP), Berlin and by the German Oilmill Association (OVID), Berlin.

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ORAL PRESENTATION THEME C

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Recent advances in the development of commercial rapeseed/canola protein products

Background: Currently, there are numerous drivers increasing the demand for vegetable proteins. In particular, the food industry is seeking nutritious, low allergen protein ingredients from sustainable and non-GMO sources. Canola proteins can meet all of these criteria and have long been of interest for use as food ingredients. However, preparation of commercially acceptable canola protein products has proven a challenge as canola seeds contain phenolics and other compounds that can negatively impact the sensory properties of the final protein products. It is of interest to advance current methods of protein extraction and purification to provide improved products and satisfy the current demand for alternative vegetable proteins.

Objectives: A first objective was to develop a commercially viable canola protein extraction and purification process to obtain isolates having improved flavour and colour profiles when compared to isolates obtained from traditional extraction and purification methods. The second objective was to ensure that the newly developed canola protein isolates possess functional and nutritional characteristics that address the requirements of food manufacturers.

Methods: Protein products were prepared from low-temperature desolventized canola meal. This specially prepared meal was extracted using a saline solution and the protein extract purified by a membrane process. The purified protein solution can either be dried to form a canola protein isolate or further processed by a dilution step to form a supernatant-derived canola protein isolate and a protein micellar mass-derived canola protein isolate.

Results: Canola protein isolates prepared from low temperature desolventized meal were found to be improved in colour and flavour over isolates made by previous methods. The purification process could be run to produce a protein isolate (trade name Nutratein™) comprised of a mixture of globulin (cruciferin) and albumin (napin) proteins. Alternatively, the purification process could be run to separate the globulins and albumins to produce a cruciferin-rich protein isolate (trade name Puratein®) and a napin-rich protein isolate (trade name Supertein™).

Nutratein™ has very good solubility across a broad pH range and has an excellent amino acid profile. It may be used to provide protein fortification in nutritional applications. Puratein® has excellent emulsifying, gelling and binding properties and may be used in applications such as dressings, meat analogues or baked goods. Supertein™ is highly soluble and has good foaming properties. It is valuable for use in applications such as beverages and aerated desserts. Supertein™ has a very high content of sulfur containing amino acids and so also may be particularly useful for functional foods.

Conclusions: A protein extraction and purification process involving low temperature desolventized meal, salt extraction, membrane and micelle technology was used to produce three canola protein isolates having colour and flavour properties improved over isolates made by past methods. The functional, nutritional and sensorial properties of these canola protein isolates open up new opportunities for use of canola proteins in food applications.

Genetic variation and inheritance of phytosterol and oil content in winter oilseed rape *(Brassica napus L.)*

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Background: Phytosterols are natural constituents of vegetable oils with serum cholesterol lowering properties. Among vegetable oils, oilseed rape is ranked the second highest in phytosterol content after corn oil. Previous studies have shown that there is a close negative correlation between erucic acid and phytosterol content, explaining the inherently high phytosterol content observed in canola quality oilseed rape (Amar et al. 2008ab, 2009). On the other hand, highly contrasting phytosterol and oil contents were found in a collection of canola quality winter oilseed rape cultivars (Amar 2009) - "Sansibar" had the highest total phytosterol content (~480 mg 100 g-1seed) and the lowest oil content (43%) while "Oase" had the lowest total phytosterol content (~360 mg 100 g-1seed) and highest oil content (46%).

Objectives: To analyze the genetic variation and inheritance of phytosterol and oil content in the winter oilseed rape DH population "Sansibar" x "Oase".

Methods: The DH population of 226 DH lines were tested at six environments in Germany and Sweden. A genetic map was constructed based on a total of 1642 markers, organized in 23 linkage groups, and covered a map length of 2350 cM with a mean marker interval of 2.0 cM. Phytosterols and fatty acids of the seed were quantified with gas-liquid chromatography. Seed oil content was determined with NIRS. QTL analysis was performed with multiple interval mapping. Identification of possible candidate genes underlying the QTL was performed by aligning the genetic map to the physical maps of Brassica rapa and Brassica oleracea.

Results: Broad-sense heritability estimates for all traits ranged from 0.84 to 0.90. Positive and highly significant correlations were observed between total phytosterol content and oil content ($r^2 = 0.24$), and between oil content and oleic acid (18:1, $r^2 = 0.48$). Between 1 and 6 QTL for phytosterols and fatty acids and six QTL for oil content were identified. With a good collinearity between genetic and physical map positions, candidate genes underlying major QTL (R2 ≥ 25%) were identified: QTL for brassicasterol on A04 was colocalized with *CYP710A1*, QTL for campesterol:sitosterol ratio and 24-methyl:24-ethyl sterol ratio on A06 were colocalized with SMT2, QTL for 18:1 and 18:3 on A01 were colocalized with FAD2 and QTL for 16:0 on A09 was colocalized with FATB.

Conclusions: Our results suggest that increasing both phytosterol and oil content is possible in canola winter oilseed rape. Major QTL corresponding to potential candidate genes could be useful for enhancing oil content and modifying the composition of phytosterols and fatty acids.

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ORAL PRESENTATION THEME C

Properties of napin from a scaled-up canola meal fractionation process

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Background: Seed storage proteins of crucifers are comprised of 12S cruciferin and 2S napin. Crucifer 2S proteins are reported to have diverse properties such as anti-microbial (Ye and Ng, 2009) and immunogenic (Barciszewski et al., 2000). Two polypeptide chains linked with four disulphide bonds (2 intra- and 2 inter-chain) compose the napin molecule (12-16 kDa) which is arranged as four alpha helices in the secondary structure. The scalable protein fractionation process developed by Agriculture and Agri-Food Canada allows obtaining napin in a highly purified form and the process is applicable to other crucifer oilseeds. Characterization of this napin protein and identification of it's distinct properties enable directing this protein product towards suitable applications.

Objectives: Investigate physico-chemical and functional properties of napin protein product obtained from canola seed protein fractionation.

Methods: Commercial *Brassica napus* canola seeds (1 mT) were processed to obtain desolventized meal under low temperature. Meal protein fractionation was carried out at pilot scale according to Wanasundara & McIntosh (2013). Spray dried napin protein isolate (NPI) was used for investigating solubility, emulsifying, foaming, heat-induced gel formation, and mineral flocculating ability and compared with commercial whey protein isolate (WPI) where applicable.

Results: Selective solubility of napin proteins in low pH aqueous medium allowed their separation from rest of the canola meal proteins. Further concentration and separation of low molecular weight components generated a napin isolate having 93.5% protein on dwb, recovering ~15.6% of meal protein in the product. The spray dried NPI was light in weight and had bulk density of 0.19 g/mL compared to commercial whey protein isolate (0.39 g/mL). The solubility properties of NPI were comparable with the WPI between pH 4 and 10 exhibiting 83-95% solubility. Among the interfacial activities, air-water interface stabilization is a significant property that NPI can provide and gave 85-90% forming ability in the pH range of 4 to 10 which is not common among plant proteins. NPI showed comparatively less effective oil-water interface stabilization ability than WPI. Moisture-free napin gave initial thermal transition at 53 °C and then another at 156 °C. NPI formed very weak heat-induced gel networks. The ability of NPI to coagulate synthetic (1% w/v) clay solutions effectively by bringing down turbidity below 100 NTU could be a valuable property to use it as a flocculating agent.

Conclusions: Separation of napin from canola meal can be achieved by simple means. Obtaining napin from *Brassica* seed meal will enable to use these proteins in various useful applications based on their interesting properties.

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ORAL PRESENTATION THEME C

Adulteration detection and authentication of rapeseed oil based on chemometric methods and fatty acid profiles

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Background: Edible oils are the most frequently counterfeited food (Moore et al. 2012). As the same as olive oil, rapeseed oils are also prone to be adulterated with the cheaper oil like soybean oil for economical reasons. Chemometric methods play an important role in the authentication identification and adulteration detection of edible oils. Since both the main edible oil and its adulterant are usually unknown, the traditional binary classification methods could not satisfy the requirement of adulteration.

Objectives: The more effective adulteration detection model should be built to quality inspection of rapeseed oil in practice. The aim of this study was to develop a robust model for authentication identification of rapeseed oils and determine the lowest detectable adulteration level (LDAL).

Methods: Random Forests (RF) and one-class partial least squares (OCPLS, Xu et al. 2011) were combined to identify the authenticity of the rapeseed oils by fatty acid profiles. Based on the previous studies (Zhang et al. 2014), 28 fatty acids were identified and quantified for rapeseed oils. Classification model was built by RF for rapeseed oil and other four kinds of edible oils. Subsequently, the OCPLS model was established. Moreover, fault oils adulterated with different levels of other edible oils were simulated by Monte-Carlo method and employed to determine the lowest detectable adulteration level of OCPLS classifier.

Results: The validation results that the RF could identify the all of rapeseed oils and OCPLS classifier could completely detect the adulterated oils and are therefore employed to authenticity assessment. The LDAL of OCPLS model was determined as 12% for rapeseed oil.

Conclusions: In this study, RF and OCPLS were combined to identify the authenticity of rapeseed oil by fatty acid profiles. The LDAL of OCPLS model was determined by Monte-Carlo method. The built model is helpful in quality inspection of rapeseed oil for protecting the customers far from adulterated rapeseed oil.

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ORAL PRESENTATION THEME C

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Genetic rescue of the lethality of *Saccharomyces cerevisiae* mutants devoid of fatty acyltransferases responsible for the initial step of glycerolipid biosynthesis by an *Arabidopsis thaliana* GPAT gene

Background: Polar glycerolipids are the primary building blocks of most membranes in living cells, and triacylglycerols (TAGs) are the most common storage form of neutral lipids serving as an energy or carbon source for a variety of cellular processes in many organisms. Despite the well-recognized importance of glycerol-3-phosphate acyltransferases (GAPTs) that catalyze the initial and committed step of glycerolipid biosynthesis, the nature or identity of the enzymes of plant origin is not well understood. Given that lipid metabolism is generally conserved between yeast and larger eukaryotes, genetic dissection of *Arabidopsis* GPATs in yeast could help unveil their functionality in glycerolipid biosynthesis, thereby moving a step closer to effective manipulation of oil biosynthesis in either seeds or vegetative plant tissues.

Objectives: We attempted to develop a robust genetic complementation system through leveraging synthetic lethality resulting from simultaneous deletion of GAT1 and GAT2 genes in yeast and to unravel the functions of the multigene family of *Arabidopsis* GPATs using this system.

Methods: Standard yeast homologous recombination and transformation were employed to create the two double conditional knockout mutants gat1Δgat2Δ(:GAL1-GAT1) and gat1Δgat2Δ(:GAL1-GAT2). To replace yeast GAT1 or GAT2 gene in the corresponding double mutant by an *Arabidopsis* GPAT gene, a shuttle vector carrying a LEU2 selection marker and an *Arabidopsis* GPAT (AtGPAT) gene was introduced into the mutants. The negative selection of the strains with 5-fluoroorotic acid (5-FOA) was then performed to remove the pYES2-GAL1-GAT1 or pYES2-GAL1-GAT2 plasmid in which the URA3 gene functions in the conversion of the nontoxic 5-FOA compound to toxic 5-flurouracil.

Results: Two conditional gat1Δgat2Δ(:GAL1-GAT1) and gat1Δgat2Δ(:GAL1-GAT2) mutants were generated. They cannot survive on 5-FOA medium, corroborating our previous finding that simultaneous inactivation of the GAT1 and GAT2 genes is lethal to yeast cells. In the presence of *Arabidopsis* GPAT1, however, their growth defect on 5-FOA can be restored. The results strongly indicate that AtGPAT1 functions in the initial step of glycerolipid biosynthesis in a similar way to yeast GAT1 and GAT2. Furthermore, we created a novel complementation system based on the gat1Δgat2Δ(:GAL1-GPAT1) strain, which possesses high specificity and robustness for characterization of the putative GPAT genes.

Conclusions: *Arabidopsis* GPAT1 behaves like yeast GAT1 and GAT2 genes with respect to involvement in mediating the initial step of glycerolipid biosynthesis. A novel genetic complementation system developed in our studies proves useful for functional dissection of a candidate GPAT gene from a eukaryote.

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ORAL PRESENTATION THEME C

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Production of "functional Oil" rich in diglycerides and phytosterol esters with enzymatic transesterification

Background: Phytosterol esters (PEs), derived from phytosterols and inheriting all of the excellent properties of phytosterols, have a much greater solubility in oils and a lower melting point as compared to the corresponding phytosterols. Furthermore, the structural and metabolic characteristics of Dglycerides (DGs) compared with triglycerides appear to be responsible for suppression of body fat accumulation, body weight loss, and lower postprandial serum triglyceride levels. Recently, Ehud et al. pointed out that PEs mixed with dietary DGs could not only influence body weight but also prevent or reverse insulin resistance and hyperlipidemia; thus, they could be serve as functional ingredients for metabolic syndrome or diabetic sufferers (Ziv et al. 2009).

Objectives: The objective of this study is to develop a novel functional oil rich in both PEs and DGs with one-pot enzymatic esterification. A rapid and convenient method was proposed for the enzymatic transesterification of phytosterols with different vegetable oils to produce functional oils in one pot.

Methods: The esterification conditions were: phytosterols (50–150 mmol/L), triglycerides such as sunflower oil, corn oil, rapeseed oil, or linseed oil (80–640 mmol/L), lipase (10–40 mg/mL), and solvent (hexane, 10 mL) were added into an Erlenmeyer flask. The vial was placed in a shaking incubator at 45–60 °C with a shaking speed of 180 rpm for a certain time. The reaction bioconversion was monitored periodically by HPLC to confirm production.

Results: Four functional oils rich in both DGs and PEs with conversions >92.1% and controllable fatty acid composition were obtained under the optimized conditions. The prepared functional oil possessed low acid value (≤1.0 mgKOH/g), peroxide value (≤2.1 mmol/kg), and conjugated diene value (≤1.96 mmol/kg) and high diglyceride and phytosterol ester contents (≥10.4 and ≥20.2%, respectively).

Conclusions: A rapid and convenient esterification method using immobilized AYS@NKA as catalyst was developed to synthesize novel functional oils rich in both PEs and DGs in high yield under mild conditions. These findings could promote the wide application of the novel functional oils produced by the food grade process in different formulations of functional foods.

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