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# **Lipids, Fats and Oils**

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## **Book of Abstracts**

**Synthesis and Applications of Sunflowerseed and Linseed Oil  
Based New Macromonomers**

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Styrene can be copolymerized with many unsaturated materials. Triglyceride oils are the renewable sources and copolymerized with styrene as well. Styrenation of drying oils and alkyds is a well-known process which has practical application in organic coatings.

In this study, we developed a novel macromonomer technique for the styrenation of sunflowerseed and linseed oils. The steps of the process are (a) alcoholysis reaction of oil with glycerol to obtain partial glycerides (PG), (b) transesterification reaction of PG with methyl methacrylate to obtain macromonomers, and (c) styrenation of macromonomers at 100°C using benzoyl peroxide as initiator.

The styrenation lead to improved film properties with triglyceride oils. Homopolymerization of the macromonomers failed most probably due to the steric hindrance of the bulky oil moieties.

## Some Compositional Components of *Rubus idaeus* L. Seed Oil

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The *Rubus idaeus* L. plant species, family Rosaceae, is a native floral element of central and eastern European regions. The fruit juice of *R. idaeus* is used in a traditional medicine, because of its cardiotoxic properties, for the improvement immune injury system and in the treatment against of the constipation. Several chemical constituent studies have been made on the carbohydrates, organic acids, vitamins and essential oils from the fruit. No medicinal use has been reported for this plant species seed oil. Thus, the seed oil composition was evaluated in the current study, which have not been previously reported.

Physico-chemical characteristic of oil sample were estimated according to the AOCS method, the fatty acids profiles were determined by GLC and the tocopherols content were analyzed by HPLC.

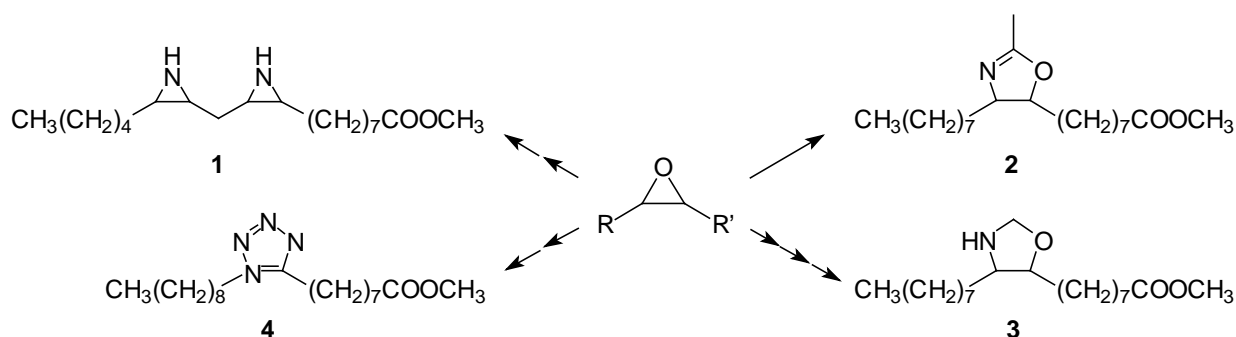
The total oil content of the seeds ranged from 12-15%. The iodine and saponification values were especially high (139 and 189), but the acid and peroxide values (1.89 and 0.22 mmolO<sub>2</sub>/kg) were relatively low. Content of the carotene was also low, meanwhile the content of chlorophyll was very high (1.73 mg/L). *R. idaeus* seed oil was characterized by a high content of polyunsaturated essential fatty acids: linoleic acid (59.2%) and linolenic acid (24.4%). Oleic acid accounted for 9.9% of the total fatty acid content. The total tocopherol content of this oil had especially high value as 358.0 mg/100g.  $\gamma$  Tocopherol (192.0mg/100g) was the major constituent, followed by  $\alpha$ -tocopherol in the yield of 154mg/100g and  $\delta$ -tocopherol in the small yield of 12.0mg/100g.

Results of the analyses of the tested seed oil indicated further use in pharmaceutical and cosmetic industries.

## Synthesis of New Heterocyclic Fatty Compounds

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Heterocyclic fatty compounds are of increasing interest, since the lipophilic alkyl chain in combination with a heterocyclic moiety is expected to possess promising biological properties. The most remarkable results were achieved by the new bisaziridine **1** derived from linoleic acid and the corresponding trisaziridine derived from linolenic acid.<sup>[1]</sup> Both compounds showed considerable cytotoxic and antimicrobial activity as well as a significant anti-tumor promoting effect.



Furthermore, we succeeded in the synthesis of the new oxazoline **2** by reaction of methyl *cis*-9,10-epoxyoctadecanoate with acetonitrile in the presence of boron trifluoride etherate. Conversion of methyl *cis*-9,10-epoxyoctadecanoate with sodium azide yielded an azido alcohol which was hydrogenated to a  $\beta$ -amino alcohol. The latter could be converted with paraformaldehyde to the oxazolidine **3**. A new 2,5-disubstituted tetrazole derivative **4** was obtained in the reaction of methyl 9(10)-oxooctadecanoate (from methyl *cis*-9,10-epoxyoctadecanoate) with sodium azide and titanium(IV) chloride in acetonitrile.

In addition, we report our results on the preparation of a large variety of 5-alkyl tetrazoles by reaction of fatty nitriles with sodium azide and triethylamine hydrochloride in toluene. Heating of the tetrazoles obtained in acetic anhydride yielded 2-methyl-5-alkyl-1,3,4-oxadiazoles. Dinitriles could easily be converted to bistetrazoles and bisoxadiazoles. N-unsubstituted tetrazoles are important in drug research since they are regarded as mimics for carboxylic acids.

[1] J.O. Metzger and S. Fürmeier, *Eur. J. Org. Chem.* **1999**, 661-664

## Antifungal effect of lauroylglycerol, lauroyldiglycerol and lauroyltriglycerol

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It is known that lauric acid and some of their derivatives have a certain level of antimicrobial activity mainly against Gram-positive bacteria, yeasts and fungi. Lauroylglycerol (monolaurin) possessing the highest antimicrobial effectiveness as well as an emulsifying activity has an excellent potential for the food production applications.

Objectives of the present study were to test the antifungal properties (inhibition of the mould colony growth rate, inhibition of the mould spore germination) of lauroylglycerol, lauroyldiglycerol and lauroyltriglycerol of the purity 99%. These substances are the surfactants with an increasing polarity (HLB values according to Davis 6.6, 8.3, 10.1) and increasing values of CMC (critical micellar concentration in water) in the range 10 – 50  $\mu\text{g}\cdot\text{ml}^{-1}$  as well. Further the effectiveness of lauroyldiglycerol and lauroyltriglycerol of the lower purity (72% for LD and 80% for LT) having significantly higher CMC values (in the range 70-100  $\mu\text{g}\cdot\text{ml}^{-1}$ ) were tested along with lauroylglycerol, lauroyldiglycerol and lauroyltriglycerol of the purity 99% against *Aspergillus* sp. DMF 0501 in the IFR gelatine cassettes and by an agar plate method.

It was found that all tested substances inhibited the germination of spores of *Aspergillus* sp. DMF 0501 and consequently suppressed the growth rate of mould colonies both in gelatine cassettes and in agar plates. The effectiveness of the spore germination inhibition decreased with the increasing polarity (HLB) of tested substances in the order: lauroylglycerol, lauroyldiglycerol and lauroyltriglycerol:

- 300 – 500  $\text{mg}\cdot\text{l}^{-1}$  (1.1 – 1.8  $\text{mmol}\cdot\text{l}^{-1}$ ) of lauroylglycerol suppressed the germination of 100 % of the present mould spores.
- 1000  $\text{mg}\cdot\text{l}^{-1}$  (2.8  $\text{mmol}\cdot\text{l}^{-1}$ ) of lauroyldiglycerol of 99% resp. 72% purity suppressed the germination of 96% resp. 50% of present mould spores.
- 1000  $\text{mg}\cdot\text{l}^{-1}$  (2.4  $\text{mmol}\cdot\text{l}^{-1}$ ) of lauroyltriglycerol of 99% resp. 80% purity suppressed only the germination of 26% resp. 11% of present mould spores.

As the trials indicated the germination of spores of *Aspergillus* sp. DMF 0501 were inhibited only when the concentration of active substances were above the level of CMC. The effect on the growth rate of mould colonies were less important, the most effective substance was lauroylglycerol.

**Development of Yellow-Seed Oilseed Rape D (*BRASSICA NAPUS* L.)**

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Above the development of different rapeseed genotypes varying in fatty acid composition, improvements of meal quality are still of importance in breeding for quality in rapeseed (*Brassica napus*). Since reduction of glucosinolates has been achieved already, the protein and crude fibre contents as well as the energy concentration of the meal have gained attention. As compared to usual black seeds yellow seediness is characterized by thinner seed coats causing a lower content of crude fibre and correspondingly higher contents of oil and protein. Regarding the distinction from domestic production of double-low rapeseed (00, Canola) yellow seed colour would have a desirable impact in both, Canola and high-erucic acid rapeseed (HEAR). In the course of a breeding program F<sub>1</sub> material was derived from crosses between dark-seeded HEAR lines and a true-breeding yellow-seeded *B. napus* (00). Both inbred and doubled-haploid (DH) lines were generated. As compared to the former, DH lines have the advantage that their selfed seed is genetically uniform and the lines are constantly reproducible. Their use for genetic studies is beneficial due to simpler gametophytic genetic ratios and more distinct phenotypic classes, which allow the scoring of rare genotypes in smaller populations. Rather than in F<sub>2</sub> populations differences caused by maternal inheritance are eliminated and analyses of recessive seed traits, such as seed coat colour, can be carried out easier. Since selection for yellow seediness is hindered due to pronounced environmental effects (e.g., temperature during seed ripening), molecular markers linked to gene loci controlling seed colour in *B. napus* have to be identified by bulked segregant analyses. In addition to visual assessment of seed colour major seed traits are analytically examined and screened by using near-infrared reflectance spectroscopy (NIRS). Results of seed quality characteristics in relation to colour will be presented.

**Molecular Breeding of Rapeseed (*Brassica Napus*)  
for modified Seed Oil Quality**

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Oil crops are important sources of energy, both for human consumption and feeding livestock. They are also sources of many non-edible purposes providing raw material for a wide range of industrial products. Rapeseed (*Brassica napus*) with Canola quality, i.e. low-glucosinolate, low-erucic varieties, nowadays represent one of the world's major sources of vegetable oil. The value of rapeseed for food and feed uses can be further improved by increasing desirable traits, e.g. oil content, and reducing undesired characteristics, e.g. fiber content or anti-nutritional compounds. Alternatives for industrial processing and non-food purposes are, for example, high-erucic acid low-glucosinolate rapeseed varieties or laurate Canola.

Biotechnological approaches combined with classical breeding schemes offer a wide spectrum of methods, such as generation of doubled-haploid lines, interspecific hybridization and molecular marker techniques, which are altogether involved in developing improved basic stocks and cultivars possessing novel desirable traits. However, further adjustments of rapeseed quality will not be realized satisfactorily without the assistance of genetic engineering. A variety of novel traits have already been introduced into rapeseed and are evaluated in field trials, including novel pollen control systems for hybrid breeding, herbicide tolerance, modified seed quality, and many others. It is expected that so-called conventional and modern methods will be used in a synergistic way in future rapeseed breeding. Corresponding recent results will be presented.

## **Genetic Modification of Sunflower Oil by Agrobacterium-mediated and Biolistic Transformation**

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In sunflower (*Helianthus annuus* L.) hybrid varieties are almost exclusively used for commercial oilseed production. Native sunflower oil is mainly used for human consumption since it contains a large amount of essential linoleic acid ( $\omega 6$  C18:2) which gives the sunflower seed oil a high nutritional value. In addition, 'high oleic' varieties have been created by mutagenesis accumulating oleic acid ( $\omega 9$  C18:1) up to 85 % in the seed oil which can be used for oleochemistry since it offers a broad range of applications. On the other hand, natural variation of important agronomic traits, such as resistances to pathogens and stresses, is rather limited although some progress has been achieved in broadening genetic variation and improvement of currently available germplasm via interspecific hybridisation. The application of genetic engineering would clearly increase the possibilities to improve the potential of this crop. In contrast to rapeseed and soybean, an efficient transformation system is lacking for sunflower due to the recalcitrance of the plant to tissue culture techniques suitable for gene transfer. Therefore, efforts regarding transformation of sunflower still cover to a large extent the improvement and optimisation of the methods for regeneration and gene transfer. Nevertheless, transgenic sunflowers have already been released to the field. Preliminary results will be reported.



**Molecular Efforts to the Biosynthesis of Medium-Chain Triglycerides in Rapeseed (*Brassica Napus*)**

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Oilseed rape (*Brassica napus* L.) is one of the leading crops which benefit from the application of genetic engineering through recombinant DNA technology. Present rapeseed oil (Canola) is characterized by a high content of unsaturated C18 fatty acids. In order to improve its industrial usefulness the project aims at the genetic modification of saturated fatty acid content and the development of transgenic *B. napus* accumulating medium-chain triacylglycerols in its seed oil. For this purpose relevant genes from unrelated plant species forming unusual storage oils are isolated and transferred to oilseed rape. Particularly in *B. napus* the efficiency of *Agrobacterium tumefaciens* mediated transformation mainly depends on the susceptibility of the starting material to agrobacteria, the ability to select for newly grown tissue derived from the transformed cells, and the potential to regenerate plants from the selected tissue. In the course of a preliminary study we have investigated intraspecific differences towards shoot regeneration by genetically transforming the resynthesized high-erucic acid rapeseed line 'RS 306' and the spring Canola cultivar 'Drakkar' with the gene construct pASBnDES1. The latter harbours a chimeric gene based on a *Cuphea lanceolata* seed-specific promoter (*CIFatB4*) and the coding sequence from rapeseed  $\Delta 9$ -desaturase in antisense orientation, in order to modify the content of oleic acid, which is the major precursor for subsequent fatty acid pathways (desaturation, elongation) in both rapeseed genotypes. First results of fatty acid patterns of transgenic plants will be presented.

### **Genetic Modification of Erucic Acid Content in Oilseed Rape**

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Breeding of high-erucic acid rapeseed (HEAR) is being focussed on the achievement of erucic acid (22:1) contents substantially higher than 45-55% in the seed oil. Rapid progress is mainly limited due to the lack of 22:1 in *sn*-2 position of the triacylglycerols (TAG) and the absence of trierucin. Molecular breeding approaches have led to the stepwise improvement of specific biosynthetic pathways in transgenic *Brassica napus*. Firstly, we have shown that down-regulation of the endogenous lysophosphatidic acid acyltransferase (LPAAT) and expression of the corresponding enzyme from *Limnanthes douglasii* R. Br. in developing rapeseeds leads to about 50% 22:1 in the *sn*-2 fatty acid composition and considerable amounts of trierucin. Secondly, with regard to the capacity of very long-chain fatty acid biosynthesis the role of the microsomal condensing enzyme ( $\beta$ -ketoacyl-CoA synthase, KCS) has been investigated by identifying and molecular characterizing more effective alleles and introducing additional KCS genes for 22:1 synthesis. The role of further enzymes putatively responsible for the improvement of 22:1 content is discussed.

## **Can hemp be a source of new oilseed, oil and food ingredients?**

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Hemp start to be recognize again as source of new oil and oilseed, this crop is known and used for centuries. Different varieties of hemp were evaluated for their composition and nutritional components. The content of nutritionally important components such as gamma and alpha linolenic acids, tocopherols and sterols at wide range were observed. Some European varieties when grown in the Canadian prairie region showed increase in the content of mentioned components by 10% to 20%. While analysis of imported hemp seeds showed lower amounts of these compounds and in some samples gamma linolenic acid was not observed while the amount of tocopherols was lower by 30% to 50%. Quality of oil produced can be related to the quality of oilseed and it maturation. Chlorophyll was mainly responsible for the stability of the oil and it quality. The amount of chlorophyll in hemp oil can range from 10 ppm to 1500 ppm and it lower value was noted at the maturity. Opposite changes were observed for the amounts of tocopherol where the highest amount was found in the mature hemp seed, while sterols content was the lowest at this stage. Quantity of amino acids were not significantly affected by maturity of the seed, however the amount of protein was lower in immaturred seeds. One of the major problems related to quality of hemp seed is the presence of THC (tetrahydrocannabinol) component treated as control substance. Work done showed that this compound is mostly in the form of a seed contaminant and the amount can be lowered by washing and/or removing hull. To conclude hemp seed can provide unique quality of oil and seed, and can be a valuable source of ingredients to be used in food application if produced from good quality seeds. Before this happen the "THC problem" have to be solved due to "zero tolerance for drugs" in many countries.

**Antioxidant Activity and Mechanism of Action of *trans*-Resveratrol in Different Lipid Systems**

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The antioxidative properties of *trans*-resveratrol were studied in the concentration range 0.02-0.2 wt % ( $8.8 \times 10^{-4}$ - $8.8 \times 10^{-3}$ M) during autoxidation at 100°C of pure triacylglycerols of lard (TGL) and of pure triacylglycerols of sunflower oil (TGSO), as well as of commercial sunflower oil. It was established that in the sunflower oil *trans*-resveratrol did not possess any antioxidative activity. In the pure lipid systems *trans*-resveratrol retarded to a large degree the oxidation process, the effect being stronger in the less oxidizable substrate, i.e. TGL. The effectiveness (stabilization factor F), strength (oxidation rate ratio ORR) and activity ( $A = F/ORR$ ) of the different concentrations of the antioxidant were determined. It was found that the activity A increased dose dependent as follows: in TGL,  $A = 3750-15425$ , and in TGSO,  $A = 79-207$ . The participation of *trans*-resveratrol in the side reactions of inhibited oxidation was discussed.

## New findings on sea buckthorn lipids

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Sea buckthorn (*hippophae rhamnoides*) is a plant known for more than two thousand years in Asia. The oil of sea buckthorn has been used in natural medicine for long times. Several positive effects to skin are well known. Nevertheless knowledge about the oil is only uncompleted. There are two oils produced from the berries – kernel oil and fruit oil. The composition of the latter one is of greater interest because of the high content of palmitoleic acid, tocopherols, carotenoids and sterols.

Data from literature vary in a wide range. This may be caused by the unknown technology or methods used for oil isolation. Today there are oils produced by solvent or supercritical extraction, by extraction with other natural oils (e.g. sunflower oil), by melting out or by enzymatic methods. Thus the obtained oils differ not only by the yield but also in the content of valuable constituents.

We used sea buckthorn oil produced from German plants (harvest 1999) produced by enzymatic fluidization of the berries. The fatty acid concentration was relatively constant over a long period and didn't strongly depend from the habitat. The concentration of palmitoleic and palmitic acid were in general higher than 30% the one of stearic acid lower than 1%.

Steroles were found in an concentration of about 0,3%. Analysis of the composition showed that  $\beta$ -sitosterole is the major constituent. The concentration of carotenoids is varying strongly. Concentration between 80 to 200 mg/kg were found. Oils produced by melting out from the dried berries shows strongly higher concentration. The oils are highly stable. Swift tests showed a induction period at 110 °C over 30h.

Further investigation were done to identify acetone insoluble matter isolated from the oil. The first results indicated only a small amount of phospholipids. A highly unpolar compound was isolated from this fraction first results indicated that this could be a highly esterified sterylglycoside. Further investigation will focus on this substance.

## **Influence of vitamin E application on phospholipid composition of muscle and on meat quality in pigs**

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The  $\alpha$ -tocopherol has been demonstrated to be the most effective of the naturally occurring tocopherols in preventing antioxidative deficiency symptoms and is the most abundant among the tocopherols in animal tissues. Vitamin E supplementation at supranutritional levels in the diet of pigs has advantages in terms of increasing the oxidative stability of phospholipids in the muscle cell membranes.

A total of 28 female pigs were fed a basal diet containing a low amount of  $\alpha$ -tocopherol (10.3 mg/kg; control), diets supplemented with 200 mg  $\alpha$ -tocopherol/ kg (group VE) or with 0.3 mg selenium/ kg (group Se) during the growing-finishing period. The vitamin E concentration of muscle was increased ( $P < 0.05$ ) immediately after slaughter and was constant up to 4 hours after slaughter in the group VE. The phospholipid composition of muscle microsomes was slightly affected by the diet. The only difference observed was in the linolenic acid level which was significantly higher in the vitamin E group. The total n-3 fatty acid concentration and the linoleic acid content of muscle mitochondria was significantly higher in vitamin E supplemented group ( $P < 0.05$ ) compared to control pigs. The peroxidative state of the animals as expressed by the malondialdehyde (MDA) concentrations of muscle immediately after death (0 h) were not affected by different dietary supplementation. Lipid peroxidation can be induced and enhanced *in vitro* by employing systems containing prooxidants like  $\text{Fe}^{2+}$ /ascorbate. TBARS of the control and Se group were increasing steadily in the 3 hours of incubation up to 2 nM MDA equivalents/mg homogenate protein. There was no increase of TBARS after stimulation of peroxidation in the vitamin E group.

Corresponding to the minor changes of the membrane fatty acid composition in microsomes the membrane fluidity, measured as the steady-state fluorescence polarisation of a lipophilic probe (1,6-diphenyl-1,3,5-hexatriene) showed no significant diet related differences at 37 °C, 33, 29, and 25 °C. The absence of a feeding effect in the present experiment may be contributed to the small differences in the PUFA/SFA ratio of microsome lipids.

The meat quality of pigs was not influenced by exogenous vitamin E and selenium. However, the incorporation of vitamin E into the muscle membranes could be important for muscle foods during storage or processing procedures.

## Poster II/4

### **Influence of chromium yeast on fatty acid composition in tissues and organs of fattening pigs**

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The aim of the present study was to assess the effect of organic chromium in the trivalent form supplemented to the diet at two different concentrations of 0.2 mg kg<sup>-1</sup> and 0.5 mg kg<sup>-1</sup> as chromium yeast on carcass traits and fatty acid composition of liver, heart, backfat, leaf fat and intramuscular fat of finishing pigs.

Ninety-six pigs of Polish White Landrace x Pietrain crossbreed (48 gilts and 48 barrows) were allotted to three treatments: 1) control with the basal diets for growing (25 - 65 kg body weight) and finishing period (65 - 105 kg BW); 2) 1 kg of basal diets supplemented with 0.2 mg Cr from chromium yeast, and 3) 1 kg of basal diets supplemented with 0.5 mg Cr from chromium yeast. The pigs were housed in pens (four gilts or four barrows per pen). Feed and water were available *ad libitum*.

Weights of liver and heart were not significantly affected by Cr supplementation. Backfat thickness over the shoulder as well as subcutaneous fat of ham was significantly reduced in pigs supplemented with Cr. A significant reduction in saturated fatty acids (SFA) content and an increase of polyunsaturated fatty acids (PUFA) level in liver, backfat and leaf fat was noticed in experimental groups. These results indicate that dietary supplementation of organic chromium may increase muscle and decrease fat deposition with lower content of SFA and higher content of PUFA in adipose tissues of backfat, leaf fat and liver. In intramuscular and heart fat no significant differences due to organic chromium addition were found.

## **Effect of supplemental microbial phytase and citric acid on nutrients content and fatty acid composition in colostrum and milk of sows**

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Addition of phytase into the feed mixtures can contribute to the decomposition of tertiary complex bonds of phytine acid with proteins or fats, and thus elevate their availability for pigs (Thompson, 1986). The quantity and quality of organic acids has a significant influence on digestibility and metabolism of proteins and fats in pigs (Gabert and Sauer, 1994).

The purpose of this experiment was to assess the effect of microbial phytase and citric acid addition into mixtures for sows on nutrient contents and fatty acid composition in colostrum and milk.

Studies were carried out using 60 sows of PL x PLW crossbreed divided into 5 groups, 12 animals each. Sows of group 1 (positive control) were fed with standard mixture of basic nutrients, Ca and P contents recommended by NRC (1998). Sows of group 2 (negative control) were given the mixture without inorganic phosphorus. Group 3 sows were fed with mixture similar to that for group 2 plus microbial phytase addition ( $500 \text{ FTU kg}^{-1}$  of feed), and group 4 of sows got mixture as for group 2 plus citric acid addition ( $20 \text{ g kg}^{-1}$  of feed). Sows of group 5 had their feed consisting of that as for group 2 with both phytase and citric acid addition. Colostrum was collected in 4 hours after farrowing and milk was collected at the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of lactation from 8 gilts in each group. Dry matter, fat, ash, lactose and crude protein contents according to AOAC (1984) were determined in samples. Fatty acid composition was estimated in fat using gas chromatography method.

The highest levels of dry matter and crude protein were found in colostrum originated from gilts of groups 3 and 5, the lowest in-groups 2 and 4 ( $P < 0.05$ ). No significant differences as regarding to nutritional components, caused by experimental factors were found in sow's milk. Monounsaturated acids were the major (57%), saturated ones – 32% and PUFA – about 11% in colostrum fat. The percentage of monounsaturated acids and PUFA decreased and that of saturated ones increased in milk fat. Addition of phytase (group 3) or phytase with citric acid (group 5) caused significant increase of PUFA content, especially those from n-6 group in colostrum fat. The tendency to alter those acid percentage in-groups 3 and 5 was also observed in milk.



## **Change of Quality Characteristics of Sunflowerseed During Ripening**

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Quality and technical-technological characteristics of seed are of special importance for sunflower processors. Seed quality - oil content and biological value - are important in order to achieve adequate quality of edible oil. The technical-technological characteristics of seed affect the processing efficiency and economic moment.

The paper presents the qualitative characteristics of seed of seven different Yugoslav sunflowerseed hybrids, from the same locality, during ripening. The dynamics of water loss and oil synthesis as well as the content of  $\alpha$ -tocopherols and waxes was followed. The technical-technological characteristics were also determined: mass of 1000 grains, liter and specific mass, hull/kernel ratio, hull thickness and seed dimensions.

The results showed that the change of technical-technological characteristics of hybrids adequately follows the dynamics of oil synthesis. The oil content of oil and  $\alpha$ -tocopherols of the investigated sunflowerseed hybrids is very high, however, the technical-technological characteristics are differing.

## **Lard as a Substrate for Following of Frying Process**

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A series of peanut frying was performed in a commercial laboratory frying-pan. Frying was performed at  $165\pm 5\text{C}^\circ$  for 30 h using different frying media (sunflowerseed oil, hydrogenated vegetable fat). Quality changes of frying media were followed determining the FFA content and dielectric constant (Food Oil Sensory - FOS value). The prooxidative value of the used medium was evaluated after 10, 20 and 30 hours of frying by addition of frying fat to lard as substrate. Oxidative stability (Rancimat and Oven test) was determined in prepared samples. In this way, in relatively short time (about 10 hours) the oxidative changes of frying medium can be estimated.

## Poster II/8

### **Influence of breed and feeding on lipid composition of beef muscle**

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The aim of this project was to increase the nutritional value of beef by enriching its n-3 fatty acid content.

Animals from two different breeds (German Holstein and Simmental) were kept either on pasture (extensive feeding) or in stable (intensive feeding). For each group we determined the intramuscular fat content of *longissimus muscle* and the fatty acid composition of the total muscle lipids. In addition we also investigated the fatty acid composition of the feed.

A significant difference in the intramuscular fat content between the two breeds was found with the German Holsteins showing a higher fat content than the Simmentals. According to the lower intramuscular fat concentration of Simmental bulls the total PUFA percentages, concerning n-3 fatty acids as well as n-6 fatty acids, is higher compared to the German Holstein breed.

The extensively kept animals proved to have a higher linolenic acid (n-3) content correlating to the large amount of linolenic acid found in grass. In contrast to this, the animals kept in stable had a higher content of linoleic acid (n-6) corresponding to the large amount of linoleic acid found in maize silage and the pellets used as feed.

In conclusion, keeping cattle on pasture leads to an increase in the n-3 fatty acid content of the meat.

Further investigations will be conducted into the relationship between phospholipid composition and volatile compounds, which are responsible for the flavour of beef.

## Comparison of the Antioxidant Properties of Different Derivatives of Cinnamic Acid on Lipid Autoxidation

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The antioxidant effectiveness, strength and activity for seven (prenyl- and methoxy-) derivatives of cinnamic acid have been compared at 0.1 mM on the kinetics of lipid (sunflower oil triacylglycerols) bulk phase oxidation by atmospheric oxygen at 353 K. Four new derivatives of cinnamic acid, 3-prenyl-4-hydroxy-cinnamic acid (I), 3,5-diprenyl-4-hydroxy-cinnamic acid (II), 2,2-dimethyl-6-carboxyethoxyethenyl-2H-benzopyran (III), 2,2-dimethyl-6-carboxyethenyl-8-prenyl-2H-benzopyran (IV) have been tested. It was established that (I) had the strongest antioxidant effect on lipid autoxidation. Neither (III), nor (IV) showed any antioxidative properties. The results obtained were compared with those of related compounds with a cinnamic acid moiety as a structure feature - p-coumaric (V), ferulic (VI) and sinapic (VII) acids. It has been found that (I) showed a stronger antioxidant activity than (V), (VI) and (II), but its antioxidative effect was smaller than this of (VII). The antioxidative activity for the investigated compounds decreased in the following sequence VII>I>II>VI>III≈IV≈V.

**A semimicro technique to analyse fats diet incorporation in the *sn*-2 position of human serum triacylglycerols.**

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The composition of position *sn*-2 of triacylglycerols (TAGs) can be determined by reacting them with the enzyme pancreatic lipase, because of its nearly absolute action for primary ester groups of TAG.

Technical procedure have been developed for 50mg - 1g sample sices. A scale which however is not adapted to any biochemical investigations.

In the present study, an attempt is therefore undertaken to downscale the established protocol to sample sices as obtained in biochemical investigations and fat absorption kinetic studies.

**Methods.** Procedures are described for hydrolysis of 10 mg and 1 mg TAG sample. The 1 mg sample being complemented with 9 mg TAG ballast. The fatty acids (FAs) ballast composition (medium chain FAs C8 to C10) is to be different from TAG sample.

**Results and discussion.** The technique is successfully validated for palm oil TAGs and human serum TAGs (Table 1), with respective reference FA distributions. Considering the limited value of the pancreatic lipase hydrolytic method for TAGs containing significant quantities of long chain polyunsaturated fatty acids, the micromethod can still provides reliable results in human serum TAGs fatty acids incorporation study.

**Table 1.** FAs content of *sn*-2 human serum TAGs fraction.

FA (% mol)	Sample size (mg)			
	10		1 <sup>a</sup>	
	Mean	S.D.	Mean	S.D.
16:0	14.5	0.8	14.3	0.6
16:1	5.8	0.4	5.5	0.4
18:0	2.5	0.5	2.9	0.4
18:1	43.1	1.6	42.4	1.4
18:2 n-6	22.6	3.3	23.2	1.6
18:3 n-3	0.7	0.2	0.7	0.1
20:4 n-6	1.7	0.3	2.1	0.3
22:5 n-3	0.9	0.3	0.6	0.1

<sup>a</sup> complemented with 9 mg of TAG ballast.

## **Energy Consumption in Soybean Meal Grinding**

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Most of oilseed solvent extraction facilities produce vegetable oil and meal as primary products. Before it can be commercialized, the oilseed meal must be treated in order to comply with international regulations on quality of meals. Moisture and solvent content and particle size are some of the factors that must be considered since they are included in such regulations.

In the oilseed process, the extracted meal, as it leaves the extractor itself, has a high content of solvent, usually hexane, which is removed in a process unit known as desolventizer-toaster (DT). Inside the DT, steam is used to strip the hexane off the meal. As a result of the desolventizing step, the solvent content of meal is reduced down to an acceptable level but its moisture content increases because of steam condensation, reaching values outside regulations. Also, such steam condensation produces partial agglomeration of meal particles forming "wet balls", with a particle size too big as compared to the normal commercial standards.

Usually, these two facts, moisture and particle size increases, are handled by drying and grinding the meal after it leaves the DT, with an important energy requirement. Since some of the meal particles at the DT outlet satisfy the regulations already, most of the times the meal is sieved at this point and only those particles larger than a limit size are subjected to grinding.

As the grinding energy consumption varies with moisture content and drying performance depends on particle size, there exists the possibility for interchanging the order of these two operations based on an economical analysis.

In this work, the energy required to grind samples of desolventized soybean meal at different moisture contents (between 12% and 16%) as a function of particle size, is measured. Bond's relationship is used to fit the experimental data and the work index for this material is evaluated. Also, drying curves of meal before and after grinding, are determined showing the influence of particle size on the drying kinetics.

Results show that decreasing meal moisture content reduces the energy required for grinding. On the other hand, the drying time increases with particle size. These results can be used to assess the convenience of drying before grinding or vice versa.

**Composition of lipid fraction in lamb muscles fed  
with various types of fodder**

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Studies were carried out in 1999 in two sheep-houses: in eastern and western Poland. Two groups of lamb-rams subjected to the slaughter at 25-30 kg body weight were material for studies. Lambs of group 1 were reared at mothers and fed with meadow hay and grinded oats grain with protein concentrate addition till about 70 days of life. After weaning, they were fed with full-dose mixture (6.4 MJ net energy and 173.3 g total protein) with the structural hay addition. Lambs of group 2 were fed with mother's milk during all feeding period. Young rams were fed with meadow hay with grinded oats grain between the 10<sup>th</sup> and 30<sup>th</sup> day of life. After that they obtained pasture fodder and meadow hay during the night. Samples of *Musculus longissimus dorsi* were taken after slaughter of lamb-rams. Fat acids contents were determined in lipid fraction by means of gas chromatography.

It was found, that oleic acid C 18:1 and palmitic acid C 16:0 as the unsaturated one, were the most abundant in muscles (40.32-48.58% and 23-26.64%, respectively). The type of feeding (group 1 or 2) had the significant influence on the content of the following acids: C 14:0, C 18:0, C 18:1, C 18:3, C 20:4. In rams fed with pasture fodder (group 2), saturated acids amounted to 45.75% and 50.62% of acids sum in particular sheep-houses. Differences between groups (type of feeding) were statistically significant ( $P \leq 0.01$ ). Comparative analysis of unsaturated acids levels revealed that statistically significant differences between both groups occurred only within monounsaturated ones.

**Content of fatty acids in depot and tissue fat of red-deer (*Cervus elaphus*) and roe-deer (*Capreolus capreolus*) obtained in middle-eastern Poland**

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The study material was sampled from red-deer (*Cervus elaphus*) and roe-deer (*Capreolus capreolus*) carcasses in 1997/1998 hunting season in middle-eastern Poland. The composition and content of fatty acids regarding to saturated and unsaturated (monounsaturated and polyunsaturated) ones were recorded in *musculus longissimus dorsi*, and samples of depot fat were taken from kidney fat. Fatty acids analyses were made using gas chromatograph

Among saturated acids, regardless the species and fat type, amounts of palmitic (C16:0) and stearic (C18:0) acids were the highest and they amounted to 18.68% and 29.31%, respectively. Saturated acids share in total fatty acids content estimated in tissue fat of roe-deer and red-deer was 52.12% and 43.44%, in depot fat – 59.53% and 57.24%, respectively. Great percentage of monounsaturated fatty acids whose amounts ranged from 30.96% to 34.87% was found among unsaturated fatty acids group. Polyunsaturated acids were 9-13% of total acids, with an exception of red-deer's tissue fat that reached 23.27%. Detailed statistical analysis of obtained results allow to state that species was the factor differentiating the fatty acids levels, but among unsaturated acids it refers only to polyunsaturated ones.

Fatty acids profile, high percentage of unsaturated fatty acids allow to qualify the wild-animal's meat from red-deer and roe-deer as that of great nutritional value.



**Modulation of brain lipids of rats by dietary rapeseed oil as compared to olive oil, low- and high-oleic sunflower oils and coriander oil**

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Our earlier feeding studies on rats, designed to evaluate the nutritional role of monounsaturated fatty acids, have revealed that dietary high-oleic olive oil and medium-oleic Canola-type rapeseed oil, as compared to high-oleic sunflower oil, steeply elevate the level of docosahexaenoic acid (DHA, 22:6 n-3) in phosphatidylethanolamines (PE) of heart and liver, however the proportions of arachidonic acid (AA) in these lipids are not substantially affected by the dietary oils [1]. On the other hand, dietary petroselinic ( $\Delta^6$ -*cis*-octadecenoic acid, 18:1 n-12) acid present at a high level in coriander oil was found to strongly reduce the level of AA in heart [2] and liver [2,3], apparently by inhibiting the  $\Delta^6$ -desaturase as a pseudo-product by mimicking the structure of  $\gamma$ -linolenic acid (GLA, 18:3 n-6), a precursor of AA.

In continuation of the above studies [1-3], the long-chain polyunsaturated fatty acids (LC-PUFA) in brain lipids were examined in male Wistar rats fed diets containing 120 g/kg feed of medium-oleic rapeseed oil (RAP), olive oil (OLI), conventional low-oleic sunflower oil (SF), high-oleic sunflower oil (HOS) and high-petroselinic coriander oil (COR) for 10 wk [4]. In the total lipids of brain the proportion of DHA was significantly higher after feeding OLI and RAP as compared to SF and COR. In PE and PC the proportion of DHA was slightly, yet significantly higher after feeding RAP as compared to OLI, SF and COR, which is attributed to a high level of  $\alpha$ -linolenic acid in RAP. The proportion of AA and other n-6 LC-PUFA in both PE and PC was significantly lower after feeding COR than all other oils, which is attributed to petroselinic acid having a  $\Delta^6$ -double bond that inhibits the  $\Delta^6$ -desaturase as a pseudo-product by mimicking the structure of 18:3 n-6, a precursor of AA.

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## **To get unsaturated fat acids´ extract from fish, with the method of supercritical fluid extraction**

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In last years, it is insisted that fats have important function in the formation period of cronic heart and cancer diseases. Nourishment with the food that contains essential fat acids rising considerably and this subject has great importance in food science Omega-6 ( $\omega$ -6) fat acids are known as essential, and the most famous representative of them is C 18:2 linoleic acid. Another important group of fat acids in the human nourishment is  $\omega$ -3 fat acids and the most known of them is  $\alpha$ -linolenic C 18:3.

Generally , unsaturated fat acids are known as essential and can not be synthesized by the human body. Omega-6 and omega-3 series of fat acids are essential for human body. Omega-6 series of fat acids are extracted from plant original fats but omega-3 series of fat acids are synthesized by green leafs and plactons. EPA and DHA are synthesized from  $\alpha$ -linolenic acid that synthesized by plancton. Because of that fish oil is the most important source of long-chained omega-3 fat acids . omega-3 fat acids have effects on liquid metabolism as synthesizer of eiconsanoids and also have biological effects in formation of structural basis of cell membrane .

In this study aim is to get unsaturated fat acids extract from anglers liver with the method of supercritical CO<sub>2</sub> extraction, and the effects of this method in fat extraction and quality.

In the process of study three different pressure degree (200bar, 240 bar and 280 bar), three different heat degree (35 C ,40 C and 45 C) and five different time intervals(60 min., 120 min., 180 min., 240 min., 300 min.,) are selected and the analysis are made in 45 different points. These analysis are the composition of total fat, procsit number , tiyobarbutiric acid number, iyot index and fat acids.

In the end of te analysis, we see that to get full of quality and unsaturated fat extraction from angler's liver with the method of supercritical CO<sub>2</sub> extraction the most ideal conditions are 280 bar pressure, 40 C heat and 300 minutes.

## **Fatty Acid Profile and Vitamin E Contents of Commercially Available Baby Food Products**

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The fatty acid composition and the contents of vitamin E were analyzed in two batches of 64 commercially available baby food products. They comprised vegetable products for babies from 5, 8 and 12 months and fruit and cereal products. Generally, all three groups of analyzed baby food products provided well-balanced amounts of both saturated (31 – 37% of total fatty acids) and unsaturated fatty acids, MUFA (23 – 26% of total fatty acids), PUFA (38 – 46% of total fatty acids). The P/S – ratio ranged from 1.1 in vegetable products for babies from 8 and 12 months and fruit and cereal products to 1.5 in vegetable products for babies from 5 months. The n-6/n-3 – ratio (linoleic acid/ $\alpha$ -linolenic acid) was very high in all groups of products (10:1 or higher) and in accordance with ESPGAN regulations (5:1 – 15:1).  $\alpha$ -tocopherol levels in vegetable products were 0.50 – 0.71 mg/100g (1.5 – 2.5 folds) and in fruit and cereal products 0.36 mg/100g (3 folds) higher than  $\gamma$ -tocopherol levels. The calculated average content of tocopherol equivalents (mg TE/100g) was between 0.6 – 0.8 for the vegetable products and 0.4 for the fruit and cereal products. The tocopherol/PUFA (mg/g) – ratios (2.5 – 4.4) were according to the ESPGAN recommended level in formulas ( $\geq 0.9$  mg vitamin E/g PUFA). Because of their tocopherol contents and vitamin E/PUFA – ratio baby food products are a good source of vitamin E activity in addition to breast milk or formula milk, for infants from 5 months onwards. They improve the total intake of vitamin E which plays a prominent role in the prevention of oxidation of the unsaturated bonds in fatty acids.

## Effect of dietary oil containing (n-3) fatty acids on beef lipids

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Numerous studies have pointed to the role of long chain polyunsaturated fatty acids [LCP - especially eicosapentaenoic (EPA) and docosahexaenoic (DHA)] in the prevention of coronary heart disease as well as other lifestyle diseases. Meat is the major source of LCP in countries with a low consumption of fish. Ruminant meats have a low level of these n-3 fatty acids. However, the enrichment of ruminant tissues was deemed possible when recent studies demonstrated that ruminal microorganisms did not hydrogenate LCP (e. g. EPA, DHA) to any significant extent. The effect of a daily supplement of 100 g fish oil on fatty acid composition and fat and meat quality characteristics were investigated in 20 fattening bulls. Fish oil contained a total of 20,8 % n-3 fatty acids (7,0 % EPA, 7,5 % DHA). The n-3 diet was fed for 113 d before slaughter.

The deposition of n-3 LCP was low compared with the dietary increment. Fish oil feeding did not affect the concentration of C 22:6, C 22:5 and C 18:3 in the *m. longissimus* (*m. long.*) whereas the content of EPA increased. Oil supplement further increased the deposition of *trans*-vaccenic acid and conjugated linoleic acid and lowered oleic acid. There was the same trend in the pelvic fat.

Two methods (Thiobarbituric Acid Reactive Substances [TBARS], Rancimat) were used to determine the oxidation stability of beef lipids (intramuscular fat, pelvic fat). Fish oil supplementation did not affect lipid oxidation of fresh and stored samples.

Furthermore, trained sensory panelists found no differences in sensory profiles (off-flavour/odour, juiciness, tenderness, flavour) between the treatments.

Table: Means and standard deviations ( $\bar{x} \pm s$ ) of meat quality characteristics of fattening bulls (*m. long.*, n = 10)

	Control	n-3 diet
<b>Selected fatty acids (% of methyl esters)</b>		
C 16:1	3,7 ± 0,58 <sup>b</sup>	4,0 ± 0,51 <sup>a</sup>
C 18:1 n-9, <i>cis</i>	36,6 ± 1,52 <sup>a</sup>	32,7 ± 1,48 <sup>b</sup>
C 18:1 n-9, <i>trans</i>	0,3 ± 0,04	0,3 ± 0,10
C 18:1 n-7, <i>trans</i>	1,1 ± 0,17 <sup>b</sup>	2,9 ± 0,48 <sup>b</sup>
C 18:2 9c/t11	0,2 ± 0,04 <sup>b</sup>	0,5 ± 0,08 <sup>b</sup>
C 18:3 n-3	0,2 ± 0,07	0,3 ± 0,06
C 20:5 n-3	0,1 ± 0,04 <sup>b</sup>	0,3 ± 0,03 <sup>a</sup>
C 22:5 n-3	0,2 ± 0,12	0,4 ± 0,23
C 22:6 n-3	0,1 ± 0,06	0,2 ± 0,10
<b>Lipid oxidation (TBARS, mg MDA/kg)</b>		
fresh	0,1 ± 0,02	0,1 ± 0,03
stored <sup>1)</sup>	0,6 ± 0,09	0,7 ± 0,05
<b>Sensory analysis<sup>2)</sup></b>		
flavour	3,2 ± 0,4	3,1 ± 0,3

<sup>1)</sup> 14 d of storage (oxygen-permeable packaging film; fluorescent lights [1800 lx; 4 °C])

<sup>2)</sup> Six-point scale (1-very poor, 6-very good)

Different superscripts indicate significant differences (Scheffé-test, P < 0,05)

**Transphosphatidylolation by immobilized phospholipase D in organic and aqueous media**

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Phospholipase D (PLD) is used for the transesterification of polar head groups in phospholipids on laboratory as well as industrial scales. Although the usage of the enzyme in immobilized form has many advantages, emulsion systems containing PLD in the aqueous phase and the phospholipids mainly in the organic phase have been preferred hitherto. The aim of the present research is to test the use of immobilized PLD for the transphosphatidylolation not only in the presence of organic solvent but also in purely aqueous media. The biotransformation in the absence of organic solvents might be important for the application in food and pharmaceutical industry. Under this aspect, we compared the conversion of phosphatidylcholine (PC) to phosphatidyl-glycerol (PG) by PLD from *Streptomyces sp.*, which was immobilized to two different carriers. The enzyme was covalently bound to porous aminopropyl glass (Sigma) by glutaraldehyde and to the macroporous synthetic polymer VA-Epoxy, Biosynth (Riedel-deHaën) by coupling via the oxirane groups of the support. The reactions were followed by HPTLC. Reaction products were quantified by densitometry. In diethyl ether/buffer both types of immobilized enzyme produced high yields of PG. The formation of the byproduct phosphatidic acid was insignificant if the water content of the system was reduced to the water entrapped in the pores of the support. In aqueous medium, however, the organic polymer proved to be much more appropriate as support of PLD than the aminopropyl glass, because in latter case the conversion of PC to PG was accompanied by remarkable adsorption of substrate and products. The optimal conditions for the synthesis of PG in aqueous medium were elaborated.

**Wax esters and triglycerides as storage substances in seeds of  
*Buxus sempervirens***

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In seeds of oil plants (sun flower, rape, flax) up to 40-65% of triglycerides occur as storage substances. However, in fruits of the Jojoba plant *Simmondsia chinensis*, belonging to the *buxaceae*, no triglycerides but wax ester were found as storage substances (1-3). These wax esters are mainly built of long-chain fatty acids and fatty alcohols with monoenoic structures, which thus represent as non-oxidizing components good lubricants and are also used in the cosmetic industry. It was of taxonomic interest to know whether seeds of the evergreen tree *Buxus sempervirens* growing in our latitudes and also belonging to the genus *buxaceae* contain as storage substances wax esters.

A lipid analysis showed, that seeds of *Buxus sempervirens* contain a yellowish oil which makes up for 42% of dry matter. However, this oil consists in contrast to the lipids of Jojoba fruits (*Simmondsia chinensis*) of only 3.6% wax esters. 95% of the oil consisted of storage substances usually occurring in oil seeds namely of triglycerides. The cell membrane forming phospholipids occur with 0.4% and glycolipids with 0.13%. The fatty acids pattern of these lipids corresponds to the typical fatty acid compositions of the corresponding lipid classes. In wax esters the monoenoic fatty acids prevail which are fatty acids with 16 and 18 carbon atoms. In the triglycerides dienoic and monoenoic fatty acids prevail. Glycolipids and phospholipid fractions are characterized by a high portion of dienoic, monoenoic and saturated fatty acids also with 16 and 18 carbon atoms.

This shows, that also in *Buxus sempervirens* the two key enzymes for the biosynthesis of wax esters occur (4). The low amount of wax esters occurring in *Buxus* in comparison to the content in Jojoba could be due to different exterior conditions such as light and temperature. It is known that a variation with respect to the quantitative lipid composition exist in dependence on outdoor factors (5-7).

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### **Selectivities of lipases from various crude plant extracts : application for oils and fats selective bioconversions.**

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Recently several plant extracts with known or just suspected enzymatic activities are coming under increased scrutiny, *Carica papaya* latex (CPL) being one of many examples. This crude latex exhibited a good lipase activity and a particular stereoselectivity for oils and fats bioconversions. In order to valorize other plant extracts, we evaluated the lipase activity and the fatty acid typoselectivity of crude extracts from *Euphorbia characias* latex and pineapple (*Ananas comosus*) stem, in comparison to crude extract from CPL. Fatty acid typoselectivity of CPL was also specified in interesterification reactions with homogeneous TAGs. In the latter part, the potential of using CPL as biocatalyst to discriminatory modify the fatty acid distribution of particular oils and fats, such as milk fat and copra oil, was further investigated.

**Methods** – In hydrolysis, lipases were analyzed for their hydrolytic actions on triacylglycerols (TAGs) ; activities were determined by the pH-Stat method and the fatty acid typoselectivities were characterized by studying the hydrolysis rates of various substrates containing different types of fatty acid. Throughout enzymatic hydrolysis of milk fat and copra oil, modifications in fatty acid distribution were evaluated by analyzing the residual acylglycerols and the released free fatty acids, separately, by GC. In transacylation reactions, activities were quantified by following the product synthesis (newly formed TAGs or fatty acid esters) by GC.

**Results** – Under optimal lipolysis conditions at pH 8.0, maximal activities were observed in the temperature range 45-55°C for all the crude plant extracts. The crude extract from pineapple stem exhibited very poor hydrolysis activity and the latex from *Euphorbia characias* showed a better lipase activity than CPL in hydrolysis. Moreover, lipases from *Euphorbia characias* latex showed a short-chain fatty acid preference in the hydrolysis of homogeneous TAGs. In CPL lipase-catalyzed interesterification reactions, the product synthesis decreased with increasing chain length of TAGs, except for reaction with tributyrin against trimargarin. Based on these particular lipase selectivities of CPL, the bioconversions of milk fat and copra oil with this biocatalyst (by hydrolysis or synthesis reactions) have permitted to discriminatory modify the initial lipids and obtain final products with a fatty acid distribution different than the initial distribution.

**Analysis of Lipids of Phytoplankton: The high level of icosapentaenoic acid in glycolipids of *Nannochloropsis oculata***

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The effects of changes of various nutrient conditions in culture media (nitrogen, phosphate and mineral availability) on the cellular lipid and fatty acid composition of a marine microalga *Nannochloropsis oculata* was investigated.

The total cellular lipid content remained between 5 – 7%, irrespective of the different nutrient conditions. The major lipid components of *N. oculata* under all conditions were triacylglycerols (TAG) as neutral lipids and glycolipids (GL) as polar lipids. Under complete nutrient deficiency condition, TAG was the dominant lipid among all of the components of the total lipids. The supplement of minerals, nitrogen, and phosphate induced a significant increase ( $P < 0.05$ ) in the level of GL and a decrease in that of TAG. Furthermore, GL was the dominant lipid under complete nutrient conditions, where all nutrients were sufficiently supplemented, and the level of TAG was the lowest among these conditions.

Under various conditions, 14:0, 16:0, 16:1n-7, and 20:5n-3 (icosapentaenoic acid, EPA) were the major components. With a change from nutrient deficiency condition through complete nutrient condition, both tissue levels of saturates and monoenes, such as 16:0 and 18:1n-9, rapidly decreased with the decrease of the amount of TAG, while that of EPA markedly increased with the replenishment of nutrients.

Consequently, although the total quantity of lipid in *N. oculata* did not change under different nutritive conditions, the quality of lipid changed. The tissue levels of EPA dramatically increased with improved nutrient conditions and this phenomenon may prepare *N. oculata* for the next step towards segmentation as EPA may play an important role as a cell membrane lipid.



**Isolation of a Kernel Thioesterase Gene of Oil Palm *Elaeis*  
*guineensis***

Omofefe Asemota, Farida Habib Shah, Maureen Bafor

**see Lecture on Monday, October 9, 2000, 11:30**

**(Biochemistry and Bioengineering)**

## **Developments in Analytical Methods and Management**

### **Poster IV/1**

#### **Micro-Analytical Membrane Separation Technology to Rapidly Evaluate Quality of Oils and Oils in Foods**

Virginia C. Gordon, Safety Associates, Inc.

Rapid, quantitative testing methods for oils and oils in foods are required to optimize routine quality control testing during processing and storage. Specific analytes should be quantitated using simple standardized separation and testing techniques.

A new micro-analytical membrane separation technology to measure multiple endpoints of degradation was evaluated on a series of oils, antioxidant treated oils, nuts, and snack products. Lipid peroxides, malonaldehyde, alkenals, and free fatty acids were rapidly measured and displayed as a profile providing important information on the degradation processes occurring in these materials. Key indicators of early and later degradation could be selected for better product control and shelf life.

The effect of antioxidants in the oils and snack products was evaluated. For some samples, shelf life increases more than three fold.

Routine quality testing of multiple indicators of degradation in oils and oils in finished food products can enhance quality, nutritional value, and shelf life of these foods.

## **Equivalence of packed and capillary GC columns with respect to suitability for foreign fat detection in butter using the triglyceride formula method**

J. Molquentin, D. Precht, Federal Dairy Research Centre, Kiel / Germany

The EU reference method "Detection of foreign fat in milk fat by gas chromatographic analysis of triglycerides" as well as the corresponding German DIN standard 10336 are based upon the use of packed GC columns. Alternatively, capillary columns can be used, if comparable results are guaranteed. Although suitable methods have already been published in 1994, the equivalence of both GC variants is still questioned. In current investigations 50 milk fat samples were selected by their C54 triglyceride content to cover a greatest possible range of variation and thus to represent most different fat compositions with respect to regional and seasonal influences as well as to breeds and feeding. After analysis of the triglyceride contents on two packed columns (OV-1) as well as on a short capillary column (HT-SIMDIST) none of these samples was found to contain foreign fat according to the above-mentioned EU method. Comparison of the S-values calculated from the triglyceride contents by the total formula resulted in correlation coefficients between the two packed columns and the capillary column of  $r = 0.94$  and  $0.92$ , respectively. The S-values are used for qualitative as well as quantitative foreign fat detection in the EU method. Also with the other triglyceride formulae correlation coefficients for S of about 0.9 were found. The repeatability limits ( $r$ ) of the S-values were met on all columns (10 consecutive analyses of a sample). Also the reproducibility limits ( $R$ ) of the S-values, i. e. the difference of an S-value between capillary and packed column, were exceeded by none of the 50 samples. Further, the excellent correlation of the contents of high-boiling C54 triglycerides between the two column types of  $r = 0.9993$  and  $r = 0.9995$ , respectively, is an indicator for a good performance. The absolute difference of C54 contents was 0.11 % and 0.05 %, on average respectively. All in all, it could be demonstrated that comparable quantitative results can be achieved when using capillary instead of packed columns. Nevertheless, due to the particularly subtle high temperature GC on short capillary columns, each analyst – as laid down in the EU method – has to check the equivalence of results once again.

**Identification and quantification of *trans* fatty acid positional isomers in German human milk lipids by TLC-gas liquid chromatography-mass spectrometry**

D. Precht, J. Molkentin, Federal Dairy Research Centre, Kiel / Germany

The intake of *trans*-octadecenoic acids (*trans*-C18:1) as well as of *trans*-hexadecenoic acids (*trans*-C16:1) is believed to be related with numerous physiological disadvantages such as the risk of coronary heart disease. Since most of the existing data on *trans*-C16:1 contents in human milk fat have been determined without a pre-separation by thin-layer chromatography (TLC), the gas chromatographically determined contents of *trans*-C16:1 frequently are too high due to overlaps with C17 fatty acids. On the other hand it is still impossible to completely separate the *trans*- from the *cis*-C18:1 isomers by simple GLC. Thus, only 57-77% (mean ca. 66%) of all *trans*-C18:1 isomers in human milk lipids are found by direct GLC. After pre-separation by Ag-TLC and subsequent GLC using a highly polar 100-m Sil 88 column, *trans*-C18:1 isomers could be quantified with a mean total content of  $2.40 \pm 0.60$  wt% in samples from 40 German women. For the positional isomers t4 (*trans*  $\Delta$ 4), t5, t6-8, t9, t10, t11, t12, t13, t14, t15 and t16 contents of 0.02, 0.02, 0.21, 0.37, 0.32, 0.68, 0.23, 0.15, 0.18, 0.09 and 0.14 wt% were established, with vaccenic acid being the predominant isomer. Further, small *trans*-C14:1 and *trans*-C16:1 contents of 0.08% and 0.15% on average were found. The *trans*-C16:1 positional isomers t4, t5, t6/7, t8, t9, t10 t11, t12, t13 and t14 could be quantified from 15 samples exhibiting mean relative contents of 2.6, 3.5, 7.6, 7.2, 24.7, 10.4, 10.1, 14.3, 8.4 and 11.3 % related to the total *trans*-C16:1 content, respectively. Also, the C16:1 isomer *trans*  $\Delta$ 3 could be identified occurring in traces with a mean absolute content of 2 mg/100 g fatty acids. A baseline separation of almost all *trans*-isomers of C16:1 as well as C18:1 could be achieved. Mass spectrometric analyses of FAME and DMOX derivatives allowed to identify the isomer *trans*  $\Delta$ 4 for the first time. Moreover, besides a mean conjugated linoleic acid (c9,t11) content of  $0.40 \pm 0.09$  % further 6 *cis/trans* isomers of linoleic acid with a total content of  $1.07 \pm 0.56$  % on average (w/o CLA) were determined. Further, 4 *trans* isomers of  $\alpha$ -linolenic acid could be baseline-resolved, exhibiting a total content of 0.11 %. Altogether German human milk lipids on average were found to contain  $3.81 \pm 0.97$  % TFA with a range of 2.38 - 6.03 %. Direct connections between the dietary intake of *trans*-C18:1 isomers and the composition of human milk lipids could be established.

## Determination of $^2\text{H}/^1\text{H}$ Ratios of Natural Products by on-line Gas Chromatography-Isotope Ratio Mass Spectrometry (HRGC-IRMS)

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Many years ago the detection of the characteristic difference of the  $^{13}\text{C}$  content of products from C3- and C4-plants has offered the possibility of the assignment of natural products to one of these plant groups. The progress of isotope ratio mass spectrometrical (IRMS) analysis based on the 'off-line' determinations of isotope ratios of the elements carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and hydrogen ( $^2\text{H}/^1\text{H}$ ) and later on the application of gas chromatography (HRGC) with the IRMS technique (HRGC-IRMS) to determine 'on-line'  $^{13}\text{C}/^{12}\text{C}$  ratios. Recently, further progress was achieved by the introduction of IRMS analysis of  $^{18}\text{O}/^{16}\text{O}$ . In 1999, the 'on-line' HRGC-IRMS analysis of  $^2\text{H}/^1\text{H}$  ratios became also available. Of all the elements, hydrogen has the largest naturally occurring variations in the ratio of its stable isotopes ( $^2\text{H}/^1\text{H}$ ); thus, new attractive fields of applications are opened. Initial applications have focused on compounds of interest to petroleum research and natural gas components as well as our flavor research. In this poster, we report first data on lipoxygenase-hydroperoxide lyase derived fatty acid degradation products, such as C<sub>6</sub>-aldehydes and C<sub>6</sub>-alcohols.

**Particle Size Influence on Solid's Oil Content  
Determination in Oilseed Industry**

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In oilseed industries, oil content in solid material at different points of the process is controlled by means of standardized methods of analysis (AOCS-American Oil Chemistry Society, ISO-International Organization for Standardization, FOSFA-Federation of Oils, Seeds and Fats Associations, etc.). Most of these techniques propose to carry out a solvent extraction working with samples of solid material with a maximal particle size. For that reason, samples are milled. As a result of this milling step, samples have a composite of particle sizes. This size distribution could vary with mill type, milling conditions and sample characteristics. Different particle size could produce variations in the results obtained in different laboratories upon the same samples. These different results could have important incidence in costs of raw materials and products.

This study was done to quantify the influence of variations in particle size on results of oil content. It has been used samples of seed, collets and desolventized material of soya supplied by regional oilseed industries. Determinations of oil content for each type of material in four different particle sizes were carried out. Analytical procedures normally utilized in our country are the corresponding official methods AOCS. In this work, Ac.3-44 and Ba.3-38 AOCS norms were utilized.

The analysis of particle size on oil content showed a marked increase in extractability for lower particle sizes in the seed. For collets and desolventized meal was observed the same effect but in a less notorious way.

The results of this study suggest that reducing maximum particle size consider 'acceptable' in the procedure utilized could reduce the time necessary for analytical determinations.

## Determination of the fatty acids composition of fish and marine algae after microwave-assisted extraction.

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Over the past 15 years a number of animal experiments, epidemiological investigations and double blind controlled clinical trials have confirmed the potential benefits of long chain polyunsaturated fatty acids (PUFAs)[1][2]. In particular 20:5 Omega-3 ( $\omega$ -3) (eicosapentaenoic acid, EPA) and 22:6 ( $\omega$ -3) (docosahexaenoic acid, DHA), but also other PUFAs are essential for the growth and development of animals in maricultures [3][4].

In the present study we have investigated the fatty acid composition of fish (mackerel, cod liver, and herring) and marine algae.

Lipids were extracted by microwave-assisted extraction (MAE) [5][6]. After lipid extraction the esterification was performed with trimethylsulfonium hydroxide (TMSH), and the resulting fatty acid methyl esters (FAMES) were determined by GC/FID [7]. Peak confirmation was obtained with GC/MS [8].

The results (mackerel and cod liver) were compared with those obtained after liquid-liquid extraction according to Bligh and Dyer [9].

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## Antioxidants prevent stereomutation and thioether formation during lipase-catalyzed thioesterification and transthioesterification for the preparation of uniform *cis*- and *trans*-unsaturated thioesters

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In organic chemistry and, particularly, pharmaceutical chemistry thioesters are utilized as "activated" esters. Lipase-catalyzed thioesterification and transthioesterification of saturated fatty acids and fatty acid esters, respectively, with alkanethiols in the presence or absence of an organic solvent are easy and convenient methods for the preparation of acyl thioesters [1-3]. However, the reaction of unsaturated fatty acids or fatty acid esters with alkanethiols leads to thiyl radical-induced stereomutation (*cis/trans*-isomerization) and to saturated thiol addition products (thioether derivatives) by the radical-induced addition of alkanethiols to >C=C< double bonds [4].

Thus, lipase-catalyzed preparation of acyl thioesters from unsaturated fatty acids or their alkyl esters and alkanethiols was found to be accompanied by the formation of geometrical isomers via stereomutation and of thioether derivatives, i.e. isomeric 9(10)-*S*-tetradecyl stearic acids and 9(10)-*S*-tetradecyl stearic acid tetradecyl thioesters, via addition at the olefinic bond, both induced by thiyl radicals [5]. Therefore, a method was developed in order to inhibit radical generation by the addition of antioxidants and thus prevent the formation of geometrical isomers and thioether derivatives during the lipase-catalyzed preparation of unsaturated acyl thioesters. In the presence of antioxidants such as 2,6-di-*t*-butyl-4-methylphenol (BHT) and octyl gallate thioesterification of oleic and elaidic acids with 1-tetradecanethiol as well as transthioesterification of methyl linoleate with 1-tetradecanethiol led to the corresponding geometrically uniform thioesters without radical-induced side reactions [5].

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## **Ag<sup>+</sup>-HPLC of various CLA isomers of milk fat before and after application of a CLA mixture**

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Conjugated linoleic acids (CLA) are a special fatty acid group with potential health-promoting properties (e.g. prevention of cancer and atherosclerosis, improving the immune function, and changing the body composition). At the present, several CLA products are available. The objective of this study was to investigate the effects of a duodenally infused CLA mixture on the milk fat synthesis and on the content and the distribution of CLA isomers in milk fat. Four Holstein cows fistulated with duodenal cannula were infused 200g of a CLA mixture daily into the duodenum over a period of 5 days. The CLA isomers were analysed by gas chromatography and by silver ion-high performance liquid chromatography (Ag<sup>+</sup>-HPLC) as their fatty acid methyl esters (FAME). The CLA supplement affected the milk fat causing a 40% reduction in milk fat percentage. The proportion of C16:0 and C16:1 decreased in response to the CLA mixture. In contrast, the percentage of C18:0, C18:2 and C18:3 increased due to the infusion. The CLA application increased the CLA percentage of the milk fat from 0.7% to 4.7% of FAME. All of the CLA isomers present in the CLA mixture were transferred into the milk fat. The apparent transfer efficiency of the infused total CLA isomers into the milk fat was about 22%. However, there were differences between the isomers. The transfer efficiency of the *trans*-10,*cis*-12 isomer was clearly lower in contrast to the other isomers. In conclusion, the duodenal application of CLA reduced the percentage of the *de novo* synthesized fatty acids. BAUMGARD et al. (2000) attributed the milk fat depressing effect to the *trans*-10,*cis*-12 isomer of CLA. Further studies are necessary to evaluate the specific role of the different CLA isomers.

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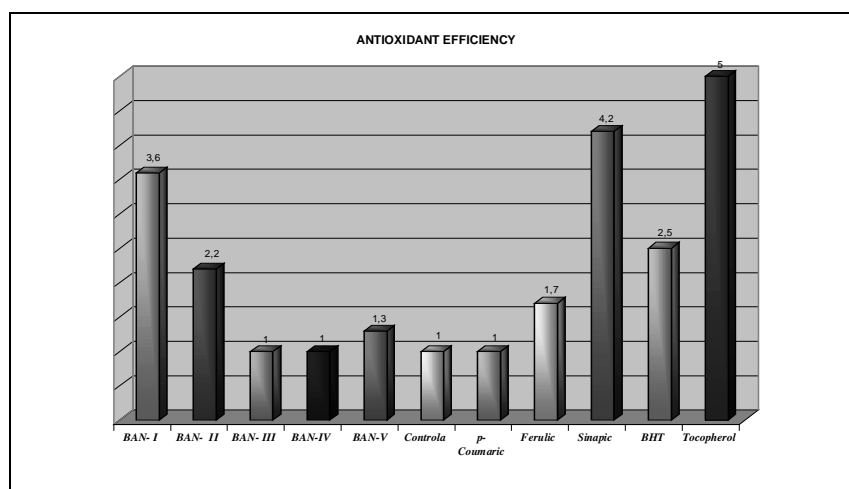
## Kinetic Analysis of Lipid Oxidation in Presence of Prenyl- and Methoxy- Cinnamic Acid Derivatives

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Antioxidant properties of 3-Prenyl-4-hydroxy-cinnamic acid (BAN I), 3,5-Diprenyl-4-hydroxy-cinnamic acid (BAN II), 2,2-Dimethyl-6-carbethoxyethenyl-2H-benzopyran (BAN III), 2,2-Dimethyl-6-carboxyethenyl-8-prenyl-2H-benzopyran (BAN IV) were compared with those of 4-hydroxy-cinnamic (p-Coumaric), 3-methoxy-4-hydroxy-cinnamic (Ferulic) and 3,5-dimethoxy-4-hydroxy-cinnamic (Sinapic) acids, and with those of standart chain-breaking phenolic antioxidants (BHT and  $\alpha$ -Tocopherol). The antioxidants were tested at 0.1 mM on the kinetics of lipid (sunflower oil triacylglycerols) bulk phase oxidation by atmospheric oxygen at 353 K.



The total ethanol extract of propolis (bee glue) from Brazil (BAN V) at 0.003wt% showed a small antioxidant activity, which strongly increased at 0.03wt%.

## Role of Phenoxyl Radicals Reactions on Methyl Linoleate Oxidation Kinetics. Computer Study

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The computer simulation of inhibited lipid oxidation is proposed for the theoretical analysis of the oxidation mechanism. The set of 22 elementary reactions similar to methyl linoleate oxidation is considered as a base kinetic model. The effect of phenolic antioxidant regeneration in phenoxyl radical reactions with lipid substrate (reaction 19), with hydroperoxides (reactions 21 and 22) and with peroxide radicals (reactions 17 and 20) is compared with those of recombination/disproportionation (reaction 16) on the kinetics of the initiated and autoxidation of methyl linoleate.

No	Reaction	Rate constants
13	$\text{PhOH} + \text{LO}_2\cdot = \text{LOOH} + \text{PhO}\cdot$	$1,5 \cdot 10^4 - 1,5 \cdot 10^6 \text{ M}^{-1} \text{ s}^{-1}$
16	$\text{PhO}\cdot + \text{PhO}\cdot = \text{P}_1 + (\text{PhOH})$	$3 \cdot 10^3 - 4 \cdot 10^7 \text{ M}^{-1} \text{ s}^{-1}$
17	$\text{PhO}\cdot + \text{LO}_2\cdot = \text{P}_2$	$3 \cdot 10^5 - 3 \cdot 10^8 \text{ M}^{-1} \text{ s}^{-1}$
19	$\text{PhO}\cdot + \text{LH} = \text{L}\cdot + (\text{PhOH})$	$0,1 \text{ M}^{-1} \text{ s}^{-1}$
20	$\text{PhO}\cdot + \text{LO}_2\cdot = \text{P}_4 + (\text{PhOH})$	$3 \cdot 10^5 - 3 \cdot 10^8 \text{ M}^{-1} \text{ s}^{-1}$
21	$\text{PhO}\cdot + \text{LOOH} = \text{LO}_2\cdot + (\text{PhOH})$	$100 \text{ M}^{-1} \text{ s}^{-1}$
22	$\text{PhO}\cdot + \text{LOOH} = \text{Q} + \text{H}_2\text{O} + \text{LO}\cdot$	$100 \text{ M}^{-1} \text{ s}^{-1}$

It is shown the great deal of the antioxidant regeneration effect in the phenoxyl radicals reactions with the oxidized substrate, with hydroperoxides and recombination /disproportionation reactions.

## The Importance of Peroxyl Radical Cyclization into Peroxide C-Radical in the Mechanism of Linoleate Oxidation<sup>#</sup>

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The kinetic and quantum chemistry methods were applied to the oxidation of methyl linoleate (LH). At 333 K, the dependence of the oxygen absorption rate ( $W_{ox}$ ) upon [LH] is non-linear whereas  $W_{ox}/(W_i)^{1/2}$  is a linear (rather than proportional) function of [LH]. These features were explained by cyclo-isomerization of the pentadienyl peroxyl radical  $LOO^\bullet$  proceeding by the intramolecular reaction of the radical centre with a double bond yielding a C-centred cycloperoxide radical ( $R^\bullet$ ) which easily turns into a new peroxyl ( $ROO^\bullet$ ).

The mechanism was supported by semi-empirical quantum chemical computations. The following energies (a probable error may reach a few kcal/mol) were obtained: 33-37 kcal/mol for  $LOO^\bullet$ , 54-56 kcal/mol for a transition state, and 17-20 kcal/mol for  $R^\bullet$ . Therefore, the cyclo-isomerization reaction is exothermal. The activation energy is 17-23 kcal/mol, which is compatible with the experimental rate constant,  $7.4 \text{ s}^{-1}$  at 333 K. The data obtained show that the postulated reaction is quite probable.

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<sup>#</sup> Dedicated to the memory of our appreciated colleague Prof. Vladimir A. Belyakov, Ph. D

(New Titel)

**Transesterification of Oil Palm Fruits using in situ Techniques**

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Research into the use of fatty acid methylesters (FAMEs) as an alternative fuel for diesel engines has continued to gain grounds especially in the developed economy. It is projected, considering attributes like biodegradability and low combustion emissions, that FAMEs will eventually replace regular petroleum diesel fuel. However, cost of vegetable oil, especially in Nigeria, imposes one of the major drawbacks on their use as fuel currently. Hence a production method that will increase the yield of FAMEs from a given quantity of seed oil can reduce this cost.

Three species - Tenera, Dura and Psifera of oil palm fruits were respectively transesterified in situ with methanol and ethanol using mineral acid as a catalyst. Yields of FAMEs from in situ transesterification were significantly greater, up to 17,5%, than those obtained from conventional reaction of pre-extracted mesocarp oil in the three reactions. Characteristics of the FAMEs produced in situ such as fatty acid composition, cloud and pour points were essentially similar to those from conventional method.

## Poster V/2

### **Improvement of the sunflower dewaxing at laboratory scale using ultrasounds**

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The sunflower oil contains waxes in variable quantity. Those waxes can be considered as crystallizable on and after 42 carbons atoms. In fact, the presence of these high melting point esters may affect the cold oil behaviour; that is the reason why they have to be eliminated. The ultrasounds may be a solution. Ultrasounds are acoustic vibrations whom the frequency is higher than 16KHz, which causes physical modifications at high power in the middle in which they spread by cavitation phenomena. Thus, thanks to their characteristics, in one hand they allow the crystals to appear and in the other hand to control their size.

The ultrasounds use parameters, which favours towards the sunflower oil dewaxing improvement are the power, the time and the frequency. The efficiency increases with the power, 50W was shown as an optimal value; the using method is a pulsation method (1s/mn); the ultrasounds application time is 15 min and more crystals are obtained with a 40KHz frequency.

The use of the ultrasounds allow to decrease the crystallisable waxes content until 47% during a close industrial dewaxing method. Thus, ultrasounds are able to improve the efficiency of the dewaxing step and so to obtain better cold quality oil. A dewaxing using ultrasounds at 30°C allow to decrease the crystallisable waxes content until 24% compare to a classical dewaxing; nevertheless, the cold test is negative (the oil sample do not stay limpid). At 20°C, the cold test is positive on the sunflower oil dewaxed using ultrasounds. Reducing energy consumption is possible through ultrasounds using (dewaxing at 20°C instead of 8°C).

## Removal of Gums and Waxes under Scrutiny

Véronique GIBON and Alain TIRTIAUX, Fractionnement TIRTIAUX, Fleurus, BELGIUM.

Dewaxing, which is a form of winterization, refers to the removal of high melting point “waxes”, which are responsible for the turbidity of some edible oils (mainly corn and sunflower) when exposed to conditions of supermarket or stored in a refrigerator.

A full dewaxing process using pressure leaf filters for the separation of the low temperature crystallized waxes can only be used on refined oils with wax content of less than 500 ppm. The dewaxing of high wax content oils is generally integrated in continuation of the neutralization step (in a chemical refining process) and is followed, depending from the cold stability requirements, by an additional polishing filtration (a possible alternative for oils of low acidity is the cold refining). The main advantage of this process lies in the fact that phospholipids, free fatty acids and waxes are removed in one combined step; the disadvantage is, besides the production of soapstocks and the important neutral oil losses, the large equipment investment as it requires at least four mixers and three centrifugal separators.

In the Super/Uni-degumming which can be used as pretreatment in a physical refining process, a part of the waxes can be removed during the hydration of the phospholipids at low temperature; this combined degumming/dewaxing requires however multi-holding steps making the process rather complicated.

The *S.O.F.T.* degumming, recently developed to achieve very low phosphorus levels in the pretreatment of physically refined oils (soybean, rapeseed, palm, ...), has been slightly modified to make possible a combination of degumming and dewaxing. The crude or water degummed oils are heated and mixed with a water solution containing chelating and wetting reagents; the emulsion is cooled to the waxes crystallization temperature and sent to a maturator. The wax crystal growth is helped by the intimate contact between the oil and the water solution of the wetting reagent; the phospholipids being transferred to the aqueous phase before crystallization starts, large crystals of waxes are formed in the maturator which are easily removed by centrifugation, giving at the end a fully degummed and dewaxed oil before entering the bleaching and deodorising/distillation units. Applied to corn and sunflower oils of different qualities, this combined process is able to properly degum and dewax (with low neutral oil losses) before bleaching, giving at the end a physically refined oil with a cold test stability of at least 48 hours at 0°C.

## Triglycerides interactions highlighted by monodirectional running on porous materials.

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For a better process and health safety, food industry must reduce the use of solvents. Among various processes, filtration was recently found as a good alternative for fat fractionation without solvent. In order to optimise the filtration process, the knowledge of the interactions between TGs and the filtration layer should be highlighted.

To avoid dynamic filtration problems, a one dimension method was used : different vegetal oils (peanut, linseed, rapeseed) were deposited at the top of a thin layer silica vertical plate. The silica was used either in the raw state or through hydrophilic (HCl) or hydrophobic (0,25 – 1 % dimethylchlorosilane) treatments. After different times of running, parallel strips of the running front were sampled and analysed by HPLC after extraction of the fat, in order to investigate the modification which may occur during migration.

A ratio front was calculated : significant TG composition differences were observed on the hydrophilic and normal silica plates. The most unsaturated TGs (LnLnLn, LLL, OLL PLL OOL) characterised by ND 4 (ND = number of double bonds) were strongly adsorbed at the origin of the plate. An average enrichment of 25 and 40% was found for normal and hydrophilic silica, respectively. Less unsaturated TGs (ND 3) (OOO, SOL) were concentrated 1,5 fold at the running front. A linear regression ( $R=0,59$ ) was found between the TG unsaturation and their composition variation between the origin of the plate and the running front. No significant variation was noted with hydrophobic modified silica. The results suggested that electrophilic interactions between TGs and support were much more important than Lifschitz - Van der Waals interactions. This assumption was confirmed by surface tension measurements ( $\text{mJ}\cdot\text{m}^{-2}$ ): i) normal silica:  $s^{\text{LW}}=53.8$ ;  $s^+=21.4$ ,  $s^-=9.6$ ,  $s^{\text{AB}}=28.7$ ,  $s^{\text{tot}}=82.6$ ; ii) hydrophilic silica :  $s^{\text{LW}}=57.6$ ,  $s^+=7.1$ ,  $s^-=76.5$ ,  $s^{\text{AB}}=46.6$ ,  $s^{\text{tot}}=102.3$ . Hydrophilic silica exhibited the most important electron donor surface tension component.

Moreover, interaction intensity was evaluated by DSC. The discrepancy between melting points ( mp ) of a pure TG and the same adsorbed on silica was related to the intensity of the interaction. mp increased with the impregnation level.



## Hydrogenation at Supercritical Single-Phase Conditions

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In hydrogenation of liquids, hydrogen is mixed with the liquid and brought in contact with a catalyst. The reaction rate is limited by the concentration of hydrogen at the catalyst surface. The reasons are the transport resistances for the hydrogen: between the gas phase and the liquid phase; through the liquid phase; and between the liquid phase and the catalyst.

By adding a suitable solvent to the reaction mixture, we can bring the whole mixture to a supercritical or near-critical state. Under these conditions the solvent dissolves both the substrate and the hydrogen and a substantially homogeneous phase is formed, and the main transport resistances are eliminated. Therefore, the hydrogen concentration at the catalyst surface can be greatly increased and extremely high reaction rates achieved. Hydrogenation at supercritical conditions can improve both the process economy and the product quality.

At this meeting we will present the basic principles for hydrogenation at supercritical conditions. We will demonstrate the principles using our results from reactions for production of hydrogenated oils and fatty alcohols.

Reaction rates up to 1000 times higher than in traditional processes have been achieved. An example of improved product quality is that in partially hydrogenated fat the *trans*-fatty acid content was dramatically reduced.

We will also say a few words about the pilot-plant that is under construction.

## Poster V/6

### **The Effects of Emulsifiers in Olive Paste**

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The emulsifiers are used to prevent the separation of oil in the olive paste due to high oil content. This study was designed to investigate the effects of emulsifiers on the characteristics of different olive pastes obtained by green olives, turning colour olives and black olives.

First, the initial characteristics of each type of olive paste were analysed, then different emulsifiers and their different percentages were applied. The physical and chemical analyses of olive pastes and their oil quality were determined and the characteristics of emulsion was studied. Moreover tasting panel was done and the olive paste were analysed according to their taste and texture.

## **Olein-Stearin Separation in the Centrifugal Field – A New Separation Technology for Dry Fractionation Processes of Fats and Oils**

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In dry fractionation processes the crystallised stearin is separated from olein by filtration with a vacuum filter or a membrane filter press. For the same separation task the use of industrial-scale nozzle separators has been demonstrated to operate reliably. A centrifuge gives more flexibility in terms of stearin hardness and olein quality during the separation process compared to a filter, which is tied to the crystallisation process. A theoretical model for the operation principle of a nozzle has been developed. The model could be verified by separation results with industrial-scale nozzle separators for palm oil and  $\omega$ -3 rich fish oil. This model allows to predict olein yield and quality for a nozzle separator by spinning a sample from the crystalliser in a lab centrifuge. For palm oil after the 1<sup>st</sup> fractionation step the olein yield is just in between the values of a vacuum and a membrane filter system. The olein quality was always slightly better compared to a membrane filter press. It could be shown that for fish oil fractionation at temperatures below 10°C an expensive installation in a cooling room is no longer required. The most practical problem in fish oil processing is polymerisation with all open filtration systems. With a hermetically closed nozzle separator of a special design this technology fulfils all requirements of a hygienic or even sterile machine with easy cleaning by automated CIP.

## Poster V/8

### **Palm oil carotene concentrate and carotene enriched palm oil**

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Palm oil has one of the highest carotene content among the various natural foods. The major carotenes found in palm oil are  $\alpha$ - and  $\beta$ -carotene. Carotenes are known to possess a number of nutritional properties such as pro-vitamin A, antioxidant and anti-cancer. There are now processes available to produce carotene enriched palm oil as well as palm carotene concentrate. The carotene enriched palm oil has 500-600 ppm of carotenes as well as 500-700 ppm of vitamin E. The palm carotene concentrate are found to contain more than 10 different types of carotenes and is available commercially. PORIM has developed processes to produce carotene enriched refined palm oil and carotene concentrate from crude palm oil. These processes were able to produce refined carotene rich palm oil as well as palm oil carotenoid concentrate.

### Exceptional lipids and fatty acids in the marine pteropod *Clione limacina*

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*Clione limacina* and *Limacina helicina* are pelagic pteropods which occur quite frequently in Arctic and Antarctic water. *C. limacina* is monophagous and feeds exclusively on *L. helicina*. The outstanding chemical features of *Clione limacina* are the extraordinary lipid and fatty acid compositions. Up to 40.6% of the total lipids consist of 1-O-alkyldiacylglycerol ethers (DAGE), a very unusual depot lipid in the marine zooplankton. In addition, the various lipid classes of *C. limacina* exhibit unique fatty acid compositions, due to high percentages of odd-chain length components, up to one third of total fatty acids. The high amounts of odd-chain length fatty acids are dominated by the 17:1(n-8) reaching 18 % followed by 15:0 and 17:0. In *Limacina helicina*, the only prey of *C. limacina*, no odd-chain fatty acids could be detected, indicating that these atypical lipids are not ingested with the prey. Major even-chain length fatty acids were 16:0, 16:1(n-7), 20:5(n-3) and 22:6(n-3). The close trophic relationships between phytoplankton - *Limacina* - *Clione* were partially reflected by the fatty acid compositions due to high amounts of 16:1(n-7), which indicate the ingestion of diatoms in *L. helicina*. The biosynthesis of odd-chain length fatty acids needs propionate as initial molecule. This may originate from dimethyl-β-propiothetin (DMPT), which has been described to be accumulated by *L. helicina* via phytoplankton uptake. These odd-chain fatty acids may serve as chemical defence agents, as indicated by their antifungal effects, a possible advantage in these shell-less pteropods, together with the production of terpenoid antifeedants.

## Initiation of lipid accumulation in fungi

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As the nutritional benefits of certain long chain polyunsaturated fatty acids are becoming appreciated so the demand for oils rich in these fatty acids is expanding. In particular, the roles of arachidonic acid [20:4n-6] (ARA) and docosahexaenoic acid [22:6n-3] (DHA) in neonate brain and nervous development has been reported and has resulted in the inclusion of these fatty acids in baby formulae. As seed oil plants do not synthesise these fatty acids, and as no amenable animal source is available, oils rich in these two fatty acids are being produced commercially using oleaginous microorganisms.

In attempts to optimise these processes and to realise new processes, a sound understanding of the biochemistry of lipid accumulation in the production organism is required. Although the biochemical basis of microbial oleagenicity has been elucidated it has been almost exclusively carried out in yeast and a similar system has tacitly been assumed to apply to other groups of eukaryotic microorganisms. However, it has become apparent that the regulation of lipogenesis differs between filamentous fungi and yeast. In this study, *Mucor circinelloides*, the commercial production organism of  $\gamma$ -linolenic acid [18:3n-6] was used as the principle model for oleaginous filamentous fungi. Another filamentous fungus, *Mortierella alpina*, due to its commercial importance, has been included in order to verify the key results.

This poster will outline a number of significant differences between the biochemistry of lipid accumulation in oleaginous yeasts and oleaginous filamentous fungi and present a revised and more concerted mechanism for the initiation of storage lipid synthesis when filamentous fungi experience N-limiting conditions

## **The *in vitro* antioxidant effect of natural carotenoid extracts on the oxidation of 10% sunflower-oil-in water emulsions**

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*Background:* Carotenoids (beta-carotene, lycopene, lutein etc.) comprise a class of natural pigments that in some cases possess a high provitamin A activity. However, nowadays, there is a growing body of evidence that these compounds could also act as antioxidants *in vitro* or *in vivo*, especially by deactivating free radicals under low oxygen pressure conditions.

*Materials-methods:* In the present project, the antioxidant activity of selected natural carotenoid extracts was studied in 10% sunflower-oil in water emulsions. Oxidation was initiated by AAPH [2,2-Azo-bis(2-amidinopropane) dihydrochloride] and lipid deterioration was monitored by UV absorbance at 234 nm and the Ferric Thiocyanate method. It was considered that the generation of AAPH water soluble free radicals would be rapid compared to the transport of oxygen through the sample, so that this system would be oxygen deficient and therefore would represent a model for the low oxygen tension conditions present in chylomicrons and other carotene containing particles *in vivo*.

*Results-Discussion:* At concentration of  $2 \text{ g l}^{-1}$  of active carotenoids (expressed as purified beta-carotene), paprika extract (containing capsorubin and capsanthin as major pigments), lipid or water preparations of annatto (containing norbixin or bixin respectively), marigold flower extract (with lutein and lutein esters) and tomato extract (lycopene as predominant pigment) were all effective as antioxidants with the order of activity being: bixin = norbixin > paprika > lutein > lycopene =  $\beta$ -carotene.  $\beta$ -Carotene was found to exert a clear antioxidant activity in proportion to its concentration ( $0\text{-}6 \text{ g l}^{-1}$ ), whereas Marigold, Paprika and annatto carotenoids markedly inhibited hydroperoxide formation although no increase in activity occurred above a specific concentration for each extract. When oxidation of the emulsions was carried out under reduced oxygen pressure (95% N<sub>2</sub>), beta-carotene was more effective than alpha-tocopherol (at 2mM). Under these experimental conditions, some decomposition of hydroperoxides also occurred, (pentanal, hexanal and 2-heptenal as major products) and headspace analysis is currently used to determine any effect of carotenoids on the formation of volatile aldehydes. Combination of carotenoid extracts ( $1 \text{ g l}^{-1}$ ) with  $\alpha$ -tocopherol ( $0.1 \text{ g l}^{-1}$ ) produced a significant increase in antioxidant activity for  $\beta$ -carotene and for lutein-rich, and paprika-rich extracts, but not for a bixin-rich extract. Carotenoid mixtures are also analysed and the one exerting the optimum antioxidant activity will be used as a dietary supplement in a future human nutrition trial.

## **Determination of Conjugated Linoleic Acid in Polish Dairy Products**

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Subject of this paper were determination of content of CLA isomers and their identification in dairy products available in Poland.

Studies were made in fat of milk and other products being an effect of its reprocessing, for example cheese.

The composition of the conjugated linoleic acid and other fatty acids were determined by GLC. The positions of the double bonds in conjugated linoleic acid were determined by mass spectrometry of dimethyloxazoline (DMOX) derivatives.

Amount of conjugated linoleic acid in fat of milk was approx 1.1% and in milk products were from 0.7 do 2.1%.



**Comparison of *trans*-Fatty Acid Composition in Partially Hydrogenated Rape Seed Oil with Use of Nickel and Palladium Catalysts**

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Subject of that was research on partially hydrogenated rape seed oil with use nickel (sample RSO-Ni) and pallad (sample RSO-Pd) catalysts.

During those researches identification and determination of geometrical and positional isomers fatty acids were processed.

RSO-Ni was achieved through hydrogenation of rape seed oil to IV 75—80 with use of nickel catalyst, including approx 22% Ni, used in fat industry, in a temperature 180—200°C, pressure 0.3MPa, amount of catalyst: 0.04% Ni.

RSO-Pd was achieved through hydrogenation of rape seed oil to IV 75—80 with use of Pd catalyst, in a temperature 160°C, pressure 2.5 MPa, amount of catalyst: 0.007%Pd (0.006%Pd supported on a fabric-formed silica).

The composition of the resulting fatty acids and content of the *trans* isomers were determined by GLC using 100 m column (SP-2560) [AOCS Official Method Ce 1f-96] and IR.

The positional of the double bonds in oleic and linoleic acid were verified by mass spectrometry of dimethyloxazoline (DMOX) derivatives.

Oleic acid positional isomers in RSO-Ni in range  $\Delta 6$ — $\Delta 16$  and in RSO-Pd in the range  $\Delta 8$ — $\Delta 16$  were determined. Amount of *trans* fatty acid isomers in RSO-Pd was approx 34% lower than in RSO-Ni.

**Study on the phytosterols composition of the oils rich  
in  $\gamma$  - linolenic acid.**

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The aim of this study was the characteristic of oils rich in  $\gamma$  - linolenic acid.

For the sterol isolation and analysis the German standard method F III (1) was used.

The method comprises: saponification of fat, isolation of unsaponifiable matter using an aluminium oxide column, separation of sterol fraction by preparative TLC and determination of the composition of sterols as trimetysilyl ethers by GLC.

The sterols content in the evening primarose, borage and black currant oil was 1,11%, 0,41%, 0,69%, respectively.

The main components of the sterol fraction were:  $\beta$ -Sitosterol, Campesterol, Methylene-cholesterol and  $\Delta^5$ - Avenasterol.

The samples of borage, evening primarose oils and black current seeds were obtained from Betuland Sp. z o.o. Warszawa

## **Synthetic Magnesium Silicate Filter Media: Effects on Frying Oil Quality and Health.**

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The 3<sup>rd</sup> International Symposium on Deep-Fat Frying held March 20-21, 2000 in Hagen-Halden, Germany, made eight recommendations regarding proper use of frying oils. Among those recommendations were: "One of the basic tools to ensure food and oil quality is the use of filtration. Filter materials should be used to maintain oil quality as needed."

Eight filter media were evaluated for their capacity to adsorb the polar compounds which result from frying oil degradation. Each of the filter media were used to treat the same degraded frying oil and the total polar materials (TPM) adsorbed were extracted with solvents from filter cakes. Diatomaceous earth showed no affinity for TPM and most of the filter media adsorbed 7 to 12% of their own weight in TPM. Magnesium silicate adsorbed 20% of its own weight in TPM. In a controlled frying study wherein fresh potatoes were fried in partially hydrogenated soybean oil, oil filtered through diatomaceous earth remained below 25% total polar compounds for 11 days. Oil treated daily with 1% by weight synthetic magnesium silicate did not reach 25% TPM until after 16 days of use. A pair feeding study showed that a diet which included highly degraded oil treated with synthetic magnesium silicate had no statistically different effect on rat liver enzymes than a diet incorporating fresh oil.

**Polymerization of Maleic Acid Modified Soybean Oil  
with Diols and Polyols**

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In this study new polymers and oligomers were synthesized from the reaction between polyols and maleinized soybean oil. The first part of this study deals with the investigation of the reaction of maleic anhydride with soybean oil. In the second part low molecular weight polyols and long chain polyols, respectively, were reacted with maleinized soybean oil.

With polyols with hydroxyl numbers between two and six, crosslinked polymers or oligomers were obtained. The effect of this functionalization on the properties and on the structure of the polymers and oligomers synthesized were studied. The product polymers were insoluble soft solids or viscous oils, having good adhesive properties and tackiness.