

**Deutsche Gesellschaft für Fettwissenschaft  
Association Française pour l' Etude des Corps Gras**

**Joint International Congress and Expo**

# **Lipids, Fats and Oils**

Opportunities and Responsibilities in the New Century  
Würzburg, Germany, October 8-10, 2000

## **Book of Abstracts**

**Monday, October 9, 2000, 8:30 (Award Lecture)**  
**Trans- and Conjugated Fatty Acids in Food –**  
**Contents and Analytical Aspects**

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The contrary effect of trans-fatty acids (TFA) and conjugated fatty acids (CFA), especially conjugated linoleic acid (CLA), which describe a group of positional and geometrical isomers of linoleic acid with conjugated double bonds, makes it advisable to gain the knowledge of the exact distribution of isomers. Most of the studies performed used a mixture of CLA isomers. To date, only the *trans*10, *cis*12 CLA isomer could be identified as changing body composition [1]. Other physiologically active CFA and the mechanism of the effects are still under discussion.

The origin of TFA and CLA in foods is partial hydrogenation, either performed industrially or biologically, the latter particularly in ruminant animals. Similar to the commercial CLA mixture the biologically formed CLA represents a mixture, too. With regard to the identification of the active isomer it is necessary to know the exact fatty-acid composition, including other conjugated fatty acids (CFA), such as conjugated C 18:3, C 20:3 or C 20:4. To analyze and/or quantify TFA or CFA isomers in foods, tissues and body liquids it is necessary to use a combination of chromatographic methods (GC and Ag<sup>+</sup>-HPLC). By application of Ag<sup>+</sup>-HPLC it is possible to quantify single CFA isomers, which partly coelute on a GC-column [2]. To confirm the position of the double bond of CFA-isomers, GC-MS is used. GC-FTIR is employed to confirm the *cis/trans* configuration.

Due to the carry-over-effect in the ruminant animals the amount of CLA is in milk and milk products (0.85 g CLA/100 g fat) and meat and meat products (0.28 g CLA/100 g fat) higher than in margarines, edible oils and frying fats (<0.01 g CLA/100 g fat). The predominant isomer in all samples is *cis*9, *trans*11 C 18:2 (rumenic acid) (approx. 80 – 90 % of total CLA) with minor amounts of other isomers. The highest TFA amount is analyzed in deep fried products (6.23 g TFA/100 g fat) and also milk products (3.94 g TFA/100 g fat). The daily CLA intake in Germany was estimated to be 0.36 g/d for women (1.9 g/d TFA) and 0.44 g/d for men (2.3 g/d) [3]. Because of the good physiological properties, CLA can probably be used in functional foods. This calls for the synthesis of selected single CLA isomers in small and higher amounts to evaluate their toxicological properties and to confirm their physiological significance. These investigations are to be the preconditions for the enrichment of CLA in foods.

- [1] Park, Y., Albright, K.J., Storkson, J.M., Liu, W., Pariza, M.W., *Lipids*, **34**(1999) 235-241
- [2] Sehat, N., Rickert, R., Mossoba, M.M., Kramer, J.K.G., Yurawecz, M.P., Roach, J.A.G., Adlof, R.O., Morehouse, K.M., Fritsche, J., Eulitz, K.D., Steinhart, H., Ku, Y., et al., *Lipids*, **34**(1999) 407-413
- [3] Fritsche, J., Steinhart, H., *Fett/Lipid*, **100**(1998) 190-210

**Monday, October 9, 2000, 9:15 (Award Lecture)**

## **Development of a Biodiesel Activity**

Georges Vermeersch, SOFIPROTEOL, 12, avenue George V – 75008 PARIS

The circumstances that led to the significant French biodiesel activity will be examined : the oilseed producers and transformers actions, the partnership with the oil refiners, the government support –regulation and detaxation.

The French production units will be described, followed by updated scientific results highlighting the advantages of biodiesel : functionality and friendly environmentally impact.

The current usage of biodiesel by oil refiners will be described and its future in the oncoming context (fuel specifications, HDI engines and post-treatment) discussed.

**Monday, October 9, 2000, 10:30 (New Generation of Products)**

**Biodiesel World 2000  
First Decennium of a Biofuel**

J.Connemann, J.Fischer, Oelmühle Leer Connemann GmbH & Co., Leer/Germany

History, background and development of a new fuel from renewable resources suitable for compression-ignition engines is presented, it is called biodiesel.

For transportation use sufficient supply of liquid energy seems necessary, existing resources of petroleum are limited and mostly located in politically rather unstable regions. Conversion technologies for coal and natural gas are not promising as additionally the development of greenhouse gases has to be controlled, CO<sub>2</sub> (carbon dioxide) is the main issue. Best solutions for the future insofar come from sun, water, wind and especially from biomass.

Oils and fats from plant or animal origin are feedstock basis for biodiesel. Though their chemistry is simple, advanced technologies and processes had to be developed. Urgent challenges of engine producers and the overall optimization of energy efficiency had to be respected mutually in process design and were met successfully.

Environmental evaluation is positive as biodiesel contains some built-in advantages for use in diesel engines, these enable e.g. efficient use of catalytic converters and deliver reduced exhaust gas emissions. Many fleet tests all over the world have confirmed suitability for diesel engines and have shown improvements of emissions and wear, so the exorbitant lubricity of biodiesel once may be seen as the most important factor. In the US it already has found entrance into legislation as an acknowledged clean alternative fuel.

Feedstock and production costs are discussed, comparisons of prices made, time frames shown in which several alternative fuels may develop. Mineral Oil taxes non-relevant for biodiesel or tax exemptions in some European countries have favoured a rather strong development of production and sales in France and Germany, Italy, Austria and Sweden. Today's high oil prices (up to 30-34 USD/bbl) additionally fire sales by positive differentials in product prices at filling stations.

This still intensifies discussions on stable and consistent qualities of biodiesel delivered by newcomers, this previewing in Germany in 1999 an Association of Biodiesel Quality Management was established to effectively control deliveries to a very sensitive market. Besides the existing national standards (E DIN 51606 in Germany) since two years an European EN standard is in preparation by several working groups of CEN/TC19 and TC307 which hopefully will be in force at mid 2001.

The upcoming question of future use of fuel cells against combustion engines is studied, impression is given that the combustion engine type will at least survive, its high efficiency and positive combination with biomass-based fuels is underlined. At the end is shown how tax exemptions for biofuels are compensated by labor and related taxes and social contributions and that they are especially justified by general transport emissions control and favorable CO<sub>2</sub> abatement and win of time.

**Monday, October 9, 2000, 11:00 (New Generation of Products)**

## **Biotechnological Production of Fatty Acid Methyl esters ("*Biodiesel*") from Deproteinised Whey**

Christoph Syldatk, Hans-Joachim Daniel, Ralf Otto, Matthias Reuss

Institute of Biochemical Engineering, University of Stuttgart

The object of this lecture is the development of biotechnological process for the production of fatty acid methyl esters ("*biodiesel*") from whey. This microbial/chemical process enables the conversion of deproteinised whey waste fractions from milk industry to products of value and at the same time the reduction of the high COD- and BOD-values of the whey. For this purpose the yeast *Cryptococcus curvatus* (old names in literature are *Candida curvata* or *Apiotrichium curvatum*) is grown on sterile filtrated whey fractions to produce intracellular triglycerides under nitrogen limitation ("*single cell oil*"). After cell disruption with glass bead mills or high pressure homogenisers and separation of the triglycerides, the fatty acid methyl esters ("*biodiesel*") are produced from these by treatment with KOH and methanol. The feasibility of this process could be shown by us in lab scale. For sterile filtration of the whey; a two step ultrafiltration process was developed. Production of *single cell oil* using the oleagineous yeast *Cryptococcus curvatus* was done in a repeated batch cultivation process enabling the production of biomass concentrations of >10% cell wet mass, which directly could be used for continuous cell disruption with a glass bead mill or a high pressure homogeniser and in a "bypass" without preceding separation or concentration of the cells.

### Literature:

Daniel, H.J., Otto, R.T., Muhm, M., Reuss, M., Syldatk, C. (1998) *Biotechnol. Lett.* 20: 805 - 807.

Daniel, H.J., Reuss, M., Syldatk, C. (1998) *Biotechnol. Lett.* 20: 1153 - 1156.

Daniel, H.J., Otto, R.T., Binder, M., Reuss, M., Syldatk, C. (1999) *Appl. Microb. Biotechnol.* 51: 40 - 45.

**Monday, October 9, 2000, 11:30 (New Generation of Products)**

**Lipase -Catalysed production of biodiesel from  
Jatropha Curcas L. Seed Oil**

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An Hsu, Jones C. Korbey, Egbe Okpefa and Jude U.Obibuzor

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PS30 lipase and a lipase from *Jatropha Curcas L.* seed were screened for their ability to transesterify *J. Curcas L.* Seed oil to its alkyl esters. (biodiesel) PS30 lipase was most efficient for converting the *J. Curcas L.* seed oil to its alkyl esters, (biodiesel). Studying the effects of different alcohols on the conversion rates of *J. Curcas* seed oil to its alkyl ester (biodiesel) using PS30 lipase, *n*-butanol gave the highest rate of 87.24% *iso*-butanol, 83.30%, *n*-propanol, 80.42%, Ethanol, 73.12%, *iso*-propanol, 49.61% and methanol, 5.76%.

The fuel properties of the *J. Curcas L.* seed oil and its biodiesel were determined. The *J. Curcas L.* seed oil had a density of 0.93, viscosity of 31.00 (mm<sup>2</sup>/sec), refractive index of 1.4070, cloud point of 0.00 degree centigrade and pour point of -9.00 degree centigrade while the *J. Curcas L.* seed oil biodiesel had a density of 0.77, viscosity, 6.54 mm<sup>2</sup>/sec, refractive index, 1.4052, cloud point, -3 degree centigrade and pour point of -6.00 degree centigrade.

**Monday, October 9, 2000, 10:30 (Human Nutrition and Animal Feeding)**

## **Biological Effects of Conjugated Linoleic Acids**

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Conjugated linoleic acids (CLA), derivatives of linoleic acid, are present in food from animal sources such as dairy food and meat. Low concentrations of CLA are found in human blood and tissues. CLA have been reported to decrease tumorigenesis in animals. They are cytotoxic to MCF-7 cells and inhibit the proliferation of human malignant melanoma and colorectal cells. Yet, CLA had no obvious effect on the growth of an established, aggressive mammary tumor in mice. Certain aspects of the immune defense were modulated, e. g. by enhanced interleukin-2 production and T cell proliferation. In hamsters CLA reduced the plasma concentrations of total cholesterol, non-high-density lipoprotein and triglycerides. These findings could not be confirmed in swine. There are also conflicting data on the effect of CLA on the development of atherosclerosis in laboratory animals. CLA rapidly reduced body fat content without affecting energy intake in mice and rats and increased the water content of the body. Moreover, CLA improved insulin sensitivity obviously by interaction with the peroxisome proliferator activated receptor- $\gamma$ . In rat hepatoma cell lines it was demonstrated that CLA are a high affinity ligand and activator of peroxisome proliferator activated receptor- $\alpha$ . The activation of peroxisome proliferator activated receptor- $\alpha$  leads to an increased expression of apolipoprotein A-I and a decreased expression of apolipoprotein C-III thereby increasing HDL cholesterol and decreasing triglycerides. Yet, an increase of HDL cholesterol was not found in laboratory animals. Moreover, the activation of peroxisome proliferator activated receptor- $\alpha$  leads to a decreased release of cytokines from smooth muscle cells derived from the aorta which is linked to slower development of atherosclerosis. CLA reduced bone formation in rats fed  $\omega$ -6 or  $\omega$ -3 fatty acids. CLA may not have antioxidant capabilities but may produce substances which protect cells from the detrimental effects of peroxides. There is, however, insufficient evidence from human epidemiological or clinical data for all these effects reported in laboratory animals. Moreover, only few of the animal studies have shown a dose-response relationship with the extent of CLA fed and biological effects. Therefore, further research is needed to clarify the role of CLA for disease prevention in humans and to show that a high intake is safe.

**Monday, October 9, 2000, 11:00 (Human Nutrition and Animal Feeding)**

## **Influence of CLA supplementation on body composition and strength in bodybuilders**

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The aim of the study was to determine the effects of a 6 month supplementation of a 7 g CLA-triacylglycerol preparation (54% total CLA) daily in comparison to a placebo oil on body composition, strength and blood parameters. 21 bodybuilders were matched into three groups: Beginners (CLA), advanced athletes (CLA), advanced athletes / beginners (placebo). Before and during the supplementation fasting blood samples were collected (start, after 3 and 6 month); the body weight and body composition were registered. Strength was measured by the one repetition maximum (1RM) of squats and bench press (start, after 2, 4, 6 month).

Results: Comparing period effects body fat decreased significantly in the treatment groups. Between the 2<sup>nd</sup> and the 3<sup>rd</sup> measurement CLA-beginners reduced their body fat from 19.4 kg to 17.6 kg. CLA-supplemented advanced athletes reduced their fat mass from 17.7 kg to 16.3 kg (2<sup>nd</sup> point) and to 15.8 kg (3<sup>rd</sup> point). Concerning fat reduction there were no significant differences compared with controls. In the group of advanced athletes (CLA) the phase angle (BIA-parameter for cell growth) increased significantly from the start (7.31°) to the third (7.82°) and to the last test (7.82 °). Testing period effects even the phase angle of the beginners and the placebo group increased significantly. Concerning the gains in strength the 1RM for squats of the advanced athletes was significantly different at any measurement point from that of the beginners and the placebo group. The results of the beginner group showed no significant differences compared with the results of the placebo group.

The CLA content of the erythrocyte membranes was not significantly different between the test groups and the placebo group for total CLA. Concerning the *trans*-10,*cis*-12-isomer at the second blood sample the beginners and advanced athletes showed a significantly higher content compared with the placebo group ( $P < 0.033 / P < 0.028$ ).

Conclusions: Even though there were significant period effects in both, body composition and strength, the results could not definitely be correlated with the CLA-supplementation, as these changes were observed in all three groups.



Monday, October 9, 2000, 11:30 (Human Nutrition and Animal Feeding)

## Substrate Selectivity of Lipases: Enzymatic Enrichment of Isomers of Conjugated Linoleic Acid (CLA)

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Federal Centre for Cereal, Potato and Lipid Research  
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In the last decade conjugated linoleic acid (CLA) – a mixture of octadecadienoic acids differing in the *cis/trans*-configuration and the position of their conjugated C=C-double bonds – has attracted considerable attention because of its anticarcinogenic, antiatherogenic and further beneficial properties. Conjugated linoleic acid can be found in meat and milk products of ruminants where the *cis-9,trans-11*-octadecadienoic acid is the predominant isomer. Moreover, CLA can also be synthesized by alkali isomerization of linoleic (*cis-9,cis-12*-octadecadienoic) acid resulting in a complex mixture of geometrical and positional (8,10-, 9,11-, 10,12- and 11,13-18:2) isomers with the two major components 9*c*,11*t*- and 10*t*,12*c*-18:2. But obviously, not all CLA-isomers have comparable physiological effects and therefore, it is important to evaluate the bioactivity of each CLA-isomer. The aim of our research is the enzymatic enrichment of isomers of conjugated linoleic acid to make them available for those studies.

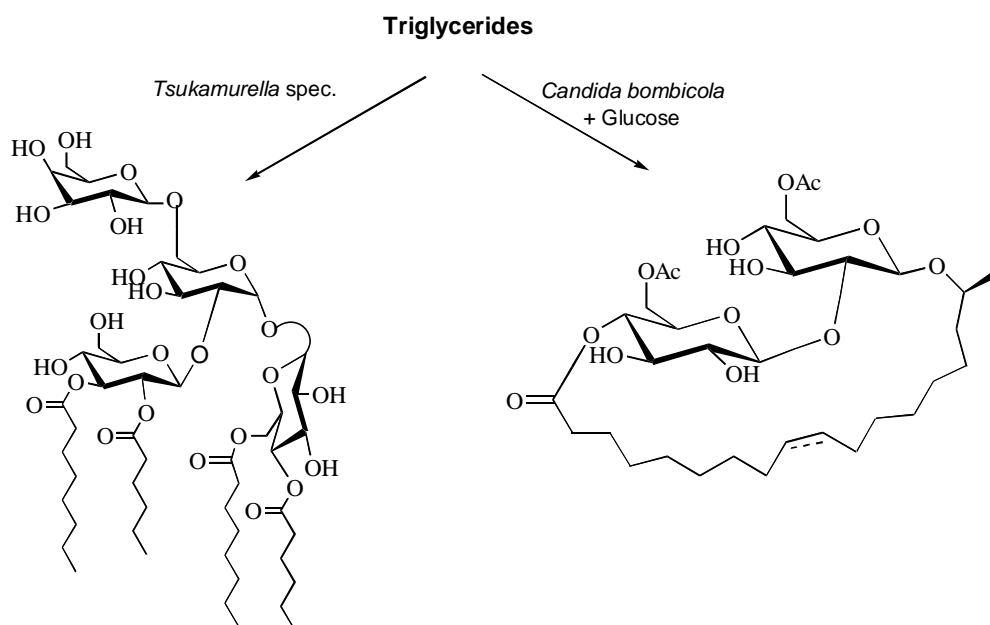
Selective lipases are used as versatile biocatalysts for the enrichment of fatty acids from fatty acid (ester) mixtures via hydrolysis, esterification and transesterification. As we could demonstrate recently in the esterification of oleic, linoleic and linolenic acid with *n*-butanol in *n*-hexane, lipases from *Candida cylindracea* and *Mucor miehei* have a high preference for fatty acids containing a (first) *cis*-double bond in  $\Delta 9$ -position. In contrast, lipase A from *Candida antarctica* (Chirazyme L-5 from Roche Diagnostics) favoured fatty acids like elaidic, linolelaidic and linolenelaidic acid with a *trans-9*-double bond. Using these biocatalysts, we examined the enzymatic esterification of the commercially available CLA-isomers 9*c*,11*t*-, 9*c*,11*c*-, 9*t*,11*t*- and 10*t*,12*c*-18:2 with *n*-butanol in *n*-hexane. As we expected, the *cis-9*-selective lipases from *Candida cylindracea* and *Mucor miehei* had a preference for the *cis-9,trans-11*-linoleic acid, while Chirazyme L-5 accepted the 9*t*,11*t*-CLA-isomer with a high selectivity. Moreover, lipase from *Candida cylindracea* as well as lipase A from *Candida antarctica* catalysed the esterification of *cis-9,trans-11*-linoleic acid with *n*-butanol faster than the corresponding reaction of 10*t*,12*c*-18:2. Because of their ability to discriminate between geometrical and positional isomers of conjugated linoleic acid, we used those lipases for the enzymatic fractionation of individual CLA-isomers, especially for the enrichment of *cis-9,trans-11*- and *trans-10,cis-12*-linoleic acid.

## Production and modification of microbial glycolipids

S. Lang, O. Langer, E. Vollbrecht, S. Hammen, R. Heckmann, U. Rau

Triglycerides are particularly appropriate carbon sources for microbial growth and biosurfactant production. The main classes of biosurfactants cover glycolipids, lipopeptides and lipopolysaccharides (Lang and Fischer, 1999; Rosenberg and Ron, 1999). During our studies on the conversion of domestic vegetable oils into added-value products we have focussed on two types of glycolipids, the structures of which (main components) are shown here:

After isolation from soil, a novel bacterium, *Tsukamurella spec.*, was able to utilize sunflower



oil for growth and additionally, to overproduce new di-, tri- and tetrasaccharide lipids. These surface-active compounds as well as their sugar backbones (after alkaline separation of fatty acids) showed interesting biological activities including antitumoral effects. Using the well-known yeast *Candida bombicola*, the cultivation conditions on glucose/rapeseed oil as carbon sources could be optimized to overproduce sophorose lipids in high quantities. Subsequently the native structure was modified by chemoenzymatic methods leading to, e.g., a new glucose lipid and an optically active (S)-17-hydroxy-*cis*-9-octadecenoic acid.

**Acknowledgement:** The authors would like to thank the German Ministry for Nutrition, Agriculture and Forestry for funds and V. Wray (GBF, Braunschweig, Germany) for molecular structure elucidation.

### References:

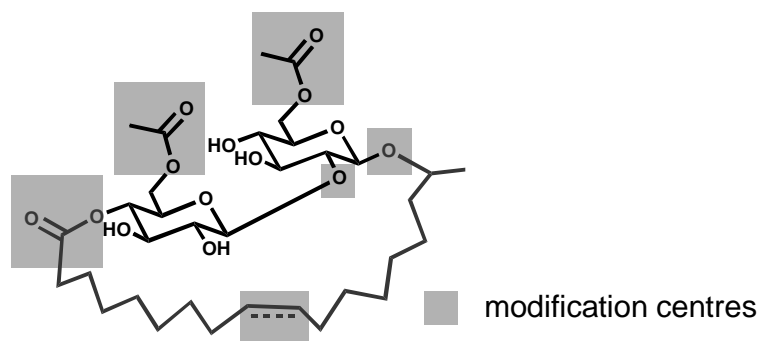
- Lang, S., Fischer, S. (1999), in: *Design and selection of performance surfactants* (Karsa, D.R., ed.), in: *Annual Surfactants Review*, Vol. 2, Sheffield Academic Press, pp. 51-103.  
Rosenberg, E., Ron, E.Z. (1999), *Appl. Microbiol. Biotechnol.* 52: 154-162.

## Sophoroselipids - A source for added value compounds

S. Hammen, R. Heckmann, S. Lang, V. Wray, U. Rau,

TU Braunschweig, Institut für Biochemie und Biotechnologie, Germany

Sophoroselipids, from *Candida bombolica* cultivated on glucose/rapeseed, are a source for preparing new compounds of useful functionality. The main component with its modification centres is presented below:



The acidic glucolipid (17-L-( $\beta$ -D-glucopyranosyl)-oxy-octadec-(9)-enoic acid) was converted into the unnatural occurring 1',6<sup>(i)</sup>-lactone with conceivable improved surfactant quality (Rau et al. 1999). The [ $\omega$ -1]-hydroxyfatty acid (17-L-hydroxy-*cis*-9-octadecenoic acid), which is commercially not available yet (Rau et al. 2000) and difficult to achieve with conventional organic methods, is a promising intermediate: cyclisation leads to macrocyclic lactones with flavour characteristics; isomerisation including modifications at the double bond yields new fatty acids. Beside the well-known surface active properties, these proposed modifications of sophorolipids opens also a field of speciality organics: chiral building blocks, flavours and fragrances.

**Acknowledgement:** The authors would like to thank the German Ministry for Nutrition, Agriculture and forestry for funding.

### References:

U. Rau, R. Heckmann, V. Wray, S. Lang (1999) *Biotechnology Letters* **21**:973-977

U. Rau, S. Hammen, R. Heckmann, V. Wray, S. Lang (2000) *J. Industrial Crops*, submitted

**Monday, October 9, 2000, 11:30 (Biochemistry and Bioengineering)**  
(was Poster III/5, did replace Lecture by Hamid and Hamid)

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

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The enzyme responsible for the hydrolysis and liberation of the acyl chain from acyl-ACP during fatty acid synthesis is the thioesterase. Investigations of thioesterase activity in plants have consistently revealed thioesterase activity specific for oleic acid, the enzyme being oleoyl-ACP-thioesterase, believed to be widely distributed in the plant kingdom.

The oil palm *Elaeis guineensis* produces two different oils in its fruits, one in the mesocarp and another in the kernel. The mesocarp oil is rich in palmitic acid (C 16.0), which may constitute up to 44% of the oil although it also contains oleic acid (C 18.1) that may make up to 35%. In the kernel, the oil consists mainly of lauric acid (C12.0), about 50%, while myristic (C14.0) and oleic acids constitute about 16% each. While the mesocarp therefore contains mainly long chain fatty acids, the kernel contains a very high proportion of medium chain fatty acids. A physical barrier, the shell, separates these two tissues in the fruit.

Oligonucleotide primers specific for conserved regions of thioesterases were used to amplify fragments of the thioesterase gene from total RNA extracted from the mesocarp of *E. guineensis*. We used the Tenera mesocarp oleoyl-ACP fragment recovered from RT-PCR, to screen an *E. guineensis* kernel library. A clone was isolated, pKTT1, that was made up of an insert of 915 base pairs. pKTT1 was sequenced and the nucleotide sequence analysis shows that it is a kernel thioesterase cDNA clone but the 5' end of the transcript is lacking. A gene bank BLAST search established the gene to be an oleoyl-ACP-thioesterase with highest homology to the *Brassica napus* oleoyl-ACP-thioesterase (E.C.3.12.14).

Although the predominant fatty acid in *E. guineensis* kernel is lauric acid, this tissue does contain about 17% oleic acid. The isolation of an oleoyl-ACP-thioesterase cDNA clone from a kernel cDNA library therefore reflects this fatty acid composition.

**Monday, October 9, 2000, 10:30 (Analytical Methods and Management)**

### **Edible oils in the test – nutritional value and oxidative stability**

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Fifty-one vegetable oils bought at small shops as well as at supermarkets were investigated. The oils differed in oil plant species (olive, sunflower, linseed, safflower and rapeseed) and in the processing (refined versus cold-pressed). The investigation criteria were the fatty acid (FA) composition (including the trans-isomeric FA), the tocopherols, the oxidative stability (acid value, peroxide value, conjugated diene value, anisidine value, induction time) and the smoke point.

The FA profile corresponded to the oilplants used. There was no difference between the refined and cold-pressed oils with exception of the higher trans-isomeric FA concentration of refined oils. But, a trans-FA content of <1 % of the total FA as well as the maximal one tenth tocopherol loss during deodorizing had no nutritional consequences. The acid value and peroxide value of refined oils was lower than of cold pressed oils. But, deodorization impaired further oxidation criteria – the conjugated dien value and the anisidine value being increased. The induction time (AOCS method Cd-b92) was only a little shortened in the cold-pressed oils.

An evaluation according to oleic acid, alpha-linolenic acid and tocopherols gives the ranking order: (1) rapeseed oil, (2) olive oil and with a certain distance sunflower oil or linseed oil. The stability criteria included olive oil comes to place *one*, rapeseed oil to place *two* the other ones rank far behind these two oils.

**Monday, October 9, 2000, 11:00 (Analytical Methods and Management)**

## **A New Method to Determine Oxidative Stability of Vegetable Fats and Oils at Simulated Frying Temperature**

S.P. Kochhar, Ch. Gertz, S. Klostermann

Good-Fry Int. n.v. R.&D. Reading,UK., Chem. Untersuchungsamt Hagen, Germany

The chemistry of vegetable oils at frying temperatures is more complex than just thermal oxidation or autoxidation. Initially the oil undergoes autoxidation, followed by thermal oxidation and reactions like hydrolysis, polymerisation and dehydration at above 150°. These reactions do not take place only in the presence of air or during the aeration with the introduction of food into the hot oil. The formation of the various decomposition products may also be catalysed by metallic ions, water, acids and alkali reacting materials. The known methods like swift-test (Rancimat) are normally executed at lower temperatures by bubbling a stream of air.

Non-refined and refined vegetable fats and oils were heated at a temperature of 170 °C after adding water-conditioned silica gel, for two hours. The degraded products were measured to assess the oil stability at frying temperature.

The determination of polymeric triglycerides by size exclusion HPLC was carried out for the estimation of the oxidative heat stability of vegetable fats and oils.

The obtained results of more than 20 different samples shows that the stability of the vegetable oils at frying temperature could not only be explained by the fatty acid composition.

There is an evidence which supports co-relationship between the unsaponifiable matter content and oxidative stability. Corn oil was more stable than soybean oil and rape seed oil better than olive oil. It was also observed that non refined oils proved to have a better stability at elevated temperature than refined oils.

**Monday, October 9, 2000, 11:30 (Analytical Methods and Management)**

## **Stabilisation of Frying Fats and Oils with Natural and Synthetic Food Additives**

Ch. Gertz, S. Klostermann, S.P.Kochhar

Chem. Untersuchungsamt Hagen, Germany, Good-Fry Int. n.v. R.&D. Reading, UK

To estimate the stabilising activity of synthetic and natural food additives at frying temperature a new method has been used (see presentation: Ch. Gertz, S.P.Kochhar, S.Klostermann "A New Method to Determine Oxidative Stability of Vegetable Fats and Oils at Simulated Frying Temperature")

Tocopherols, various tocopherol esters and lecithins, phytosterol fractions, phenolic compounds like quercetin, oryzanol, ferulic acid, squalene, BHT, BHA and other compounds like ascorbic acid 6-palmitate, gallates are added to refined sunflower and rape seed oil and their effectiveness has been investigated.

Both linoleic and oleic rich oils gave comparable results for the activity of the various compounds. Alpha tocopherol, tocopherol esters and BHA have low or negative effects at frying temperature. Ascorbic acid 6-palmitate and some phytosterol fractions were found to be the most antioxidativ compounds.

**Monday, October 9, 2000, 13:30 (New Generation of Products)**

## **Effect of structure of defined triglycerides on the physical properties of fatty bases**

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The structural triglycerides synthesising during the fat transesterification play an important role in effecting the physical properties of fatty basestocks of emulsified fats. These fatty bases contain the minimal content of trans-isomers. With respect to the fact that during transesterification of triglycerides the mixture of substances are produced, following defined chemical structures of trisaturated triglycerides were synthesised in a laboratory scale:

- 1-caprinoyl-2-stearoyl-3-lauroylglycerol  
Pairs of asymmetrical and symmetrical structures:
- 1,2-dicaprinoyl-3-stearoylglycerol a 1,3-dicaprinoyl-2-stearoylglycerol
- 1,2-dilauroyl-3-stearoylglycerol a 1,3-dilauroyl-2-stearoylglycerol
- 1,2-dimyristoyl-3-stearoylglycerol a 1,3-dimyristoyl-2-stearoylglycerol

The synthesised substances were characterised by use of the standard methods. The model fatty bases were prepared by blending of synthesised triglycerides with canola oil in the rate 10-35 : 35-65 % w/w. The crystallisation was run under the defined conditions. The fatty bases were characterised by use of the melting point, solidification point, SFC profile (p-NMR). The consistency relating parameters were read from a flow curve (by means of rotatory rheometry method in cone – plate arrangement).

On the base of results obtained the following conclusions can be done:

1. It was found that the fatty bases with the symmetrical triglycerides had always higher melting point, solidification point and SFC profile compared with asymmetrical ones.
2. The differences in the melting point, solidification point and SFC profile in the series 1,3-dicaprinoyl-2-stearoylglycerol, 1,3-dilauroyl-2-stearoylglycerol a 1,3-dimyristoyl-2-stearoylglycerol were more significant in the fatty bases with symmetrical triglycerides compared with asymmetrical ones.
3. The model fatty bases possessed the properties of viscoplastic thixotropic substances represented by the Bingham`s linear model.
4. SFC value depended nonlineary on the static yield value in the temperature range 10 – 35 °C.



**Monday, October 9, 2000, 14:00 (New Generation of Products)**

## **How to calculate phase diagrams for microemulsions**

D. Vollmer, Univ. of Mainz

For non-ionic microemulsions a simple rule is given to achieve a semiquantitative prediction of phase boundaries. In especial, we consider the phase boundaries between a single-phase microemulsion and a two-phase microemulsion (i.e. a microemulsion coexisting with a water- or an oil-rich phase), as well as between a two-phase microemulsion and a three-phase microemulsion (i.e. a microemulsion coexisting with a water- and an oil-rich phase). Our ansatz treats weak and strong surfactants within a unified approach.

**Monday, October 9, 2000, 14:30 (New Generation of Products)**

## **The influence of high pressure upon the phase behaviour of edible fats**

E. Frede, W. Buchheim, Federal Dairy Research Centre, D24121 Kiel, POB 6069

Under pressure, according to Le Chatelier-Braun's principle, phase equilibria in fats are reversibly shifted to higher temperatures. Our former investigations had mainly been focussed on pressure-induced crystallization of emulsified milk fat showing, e.g., that the well-known super-cooling of milk fat can be largely overcome by special pressure treatments. In bulk fats modified textures or other novel product properties may form under pressure. In order to estimate the technological potential of high-pressure applications to (non-emulsified) food lipid systems, the modified melting and crystallization behaviour of various edible fats and oils (e.g., milk fat, milk fat fractions, lard, coconut and rapeseed oil), food emulsifiers (acetic acid esters (ACETEM) and propylene glycol esters (PGMS) of monoglycerides and model systems (stearic and oleic acid, tristearin and triolein) was measured under pressure (0.1 ... 400 MPa).

Our high-pressure autoclave allows studying lipid samples within a plastic tube (4.5 ml volume) by a permanently inserted thermocouple. Defined heating or cooling programs (0 ..... 90°C, max. 30 K/h) can be performed under pressure at isobaric conditions. In order to emphasize the exo- and endothermic processes during heating and cooling a computerized differential method similar to that of DTA was applied. Phase transitions are then pronounced as more or less sharp peaks. By the equipment any preset pressure may be built up within a few seconds. The subsequent decompression to ambient pressure takes place at similar rates. The sample response consists of a transient increase or drop of temperature.

It could be demonstrated by our investigations that the temperature-time curves obtained after (adiabatic) compression or subsequent decompression showed characteristic courses depending on whether endo- or exothermic phase transitions had been induced. The maximal temperature change occurred approx. 30 s after compression /decompression and varied between 6 and 16 K per 100 MPa depending on the experimental conditions (temperature, pressure) and the type of lipid. The pressure-induced shifts of phase transition temperatures as derived from linear heating or cooling regimes at isobaric conditions amounted to approx. 16 K/100 MPa for most of the investigated edible fats, which ranged between oleic acid (14 K/100 MPa) and the emulsifiers studied (20 K/100 MPa). These results were based on a linear approximation of the measured transition temperature / pressure relationship ( $R^2 > 0.99$ ). A better fit of the experimental data, however, was achieved by square approaches ( $R^2 > 0.999$ ) resulting in a slight decrease of the before-mentioned (mean) values with pressure.

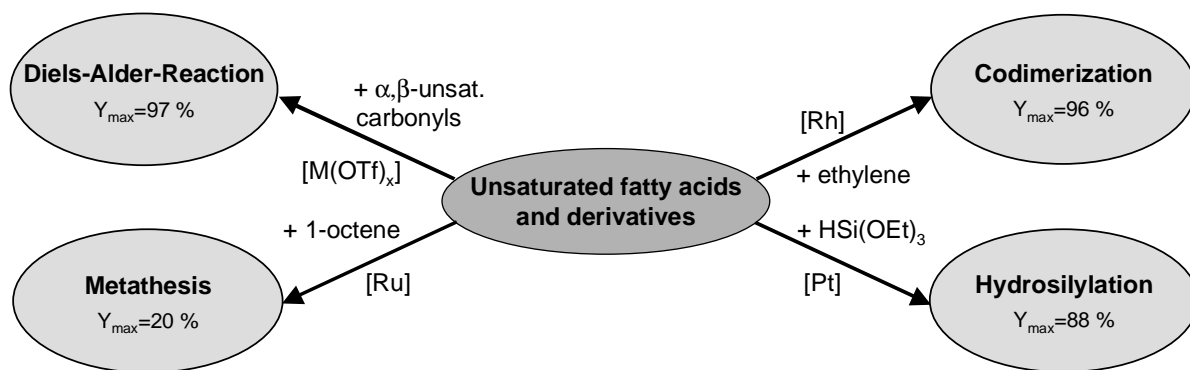
Monday, October 9, 2000, 15:00 (New Generation of Products)

## New Oleochemicals by Homogeneous Catalysis

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The derivatives of renewable raw materials – especially of fatty acids and oils – represent a very interesting class of new substances. The reactions are carried out under mild conditions and with high yields by using homogeneous transition metal catalysis. The following figure shows our research projects of exploring new catalytical syntheses of oleochemicals, which can be used for many applications. E.g. the products of the Diels-Alder-reaction can be used as starting materials for lubricants, polymers or surfactants. Internal branched fatty acids could be synthesized by codimerization of methylolinate and  $\alpha$ -olefines. Because of their excellent physical properties they can be used e.g. in lubricants or as softeners. All newly synthesised substances are expected to be biologically well decomposable.



Our aim is both the optimization of the catalytic system and the process development of new products based on renewable raw materials. Especially in the Diels-Alder-reaction of methylolinate with  $\alpha,\beta$ -unsaturated carbonyls the problematical recycling of the homogeneous dissolved catalyst could be solved by a water extraction step.

In the codimerization of methylolinate and in the hydrosilylation of methylundecenoate multicomponent solvent systems proved to be very interesting, because they guarantee a single-phase reaction process (repress of the miscibility gap at reaction temperature), whereas the catalyst and the products can easily be separated by the two-phase technique at room temperature.

We give an overview about our work applying classical transition metal catalysis on new syntheses with renewable raw materials.

**Monday, October 9, 2000, 13:30 (Human Nutrition and Animal Feeding)**

## **New Reference Values for Nutrient Intake in Germany, Austria and Switzerland**

G. Wolfram, Weihenstephan

The nutrition societies of the three countries prepared a new and the first time joint edition of reference values for nutrient intake. In this lecture the new reference values for fat intake will be discussed.

In developed countries atherosclerotic cardiovascular disease is the reason for about 50 % of all deaths. Because of the severe morbidity and high mortality primary prevention is the best strategy. Epidemiological studies show a strong, positive relationship between fat intake, plasma cholesterol concentrations and the incidence of CHD. The association between dietary intake of fat and cholesterol and the extent of atherosclerosis and CHD has been recognized in intervention studies. The amount of saturated fat in the diet correlates stronger with the incidence of CHD than total fat intake. The consumption of unsaturated fatty acids, however, appears to be beneficial, since it is inversely correlated with the plasma cholesterol concentration and risk of myocardial infarction. Additional nutritional factors like trans fatty acids with a negative influence on risk or positive factors like  $\alpha$ -linolenic acid have attracted much attention.

Excessive dietary fat intake has been linked to increased risk of obesity, coronary heart disease and certain types of cancer. The degree of risk of these and other factors may vary according to: type and level of fatty acid intakes, percentage of energy from total fat, dietary cholesterol, LDL and HDL levels, intakes of antioxidants and dietary fibre, physical activity levels and health status. Low-fat diets are often lower in cholesterol and higher in antioxidants and dietary fibre.

Active individuals who are in energy balance may consume up to 35 percent of their total energy intake from dietary fat. Sedentary individuals should not consume more than 30 percent of their energy from fat. Intakes of saturated fatty acids should provide not more than 10 percent of energy. Desirable intakes of polyunsaturated fatty acids should provide not more than 7 percent of energy. Intakes up to 10 percent energy are recommended when intakes of saturated fatty acids are more than 10 percent of energy. Reasonable restriction of dietary cholesterol (less than 300 mg/day) is advised. Food manufacturers should reduce the levels of trans isomers of fatty acids arising from hydrogenation.

The n-6 and n-3 fatty acids have critical functions in the membrane structure and as precursors of eicosanoids, which are potent and highly reactive compounds. Various eicosanoids have often opposing effects on, for example, smooth muscle cells, platelet aggregation, vascular parameters (permeability, contractility), and on the inflammatory processes and the immune system. Since they compete for the same enzymes and have different biological roles, the balance between the n-6 and the n-3 fatty acids in the diet is of considerable importance.

A number of studies have shown that the consumption of foods (such as oil-rich fish) containing the long-chain n-3 fatty acids Eicosapentaenoic acid (EPA) and DHA or  $\alpha$ -linolenic acid, is associated with decreased risk of coronary heart disease (CHD), probably because of mechanisms not related to serum lipoprotein levels. The ratio of linoleic to  $\alpha$ -linolenic acid in the diet should be below 5:1.

**Monday, October 9, 2000, 14:00 (Human Nutrition and Animal Feeding)**

## **Nutritional implication of *trans* fatty acids during perinatal period, in French pregnant women**

Boué C.<sup>a</sup>, Combe N.<sup>a</sup>, Billeaud C.<sup>b</sup>, Entressangles B.<sup>c</sup> (financially supported by ONIDOL, CSM, SGFHTF and Astra-Calvé Society).

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Some study have demonstrated the transfer of *trans* fatty acids (TFA) across the Human placenta and from maternal diet into human milk. On the other hand, it was suggested that TFA might disturb the metabolism of essential fatty acids (EFA) in fetus and consequently might affect intrauterine human growth more or less according to the TFA intake level of the mother. In this context, the objective of this study was to assess, for a French pregnant women, possible impact of their TFA intake on either the birth weight and head circumference or the TFA composition of the umbilical cord tissues (plasma lipids and parietal phospholipids) of their term infants. The TFA composition of plasma lipids and parietal phospholipids of umbilical cord were determined by gas chromatography, associated with a thin-layer chromatography for the phospholipids. Maternal milk samples (n=40), obtained 7 days after the parturition, were also analysed, using a combination of AgNO<sub>3</sub> thin-layer chromatography and capillary gas chromatography, in order to appreciate the content and origins of TFA (ruminant fats and partially hydrogenated vegetable oils).

In milk fat, the mean content of total TFA, expressed as proportion of all fatty acids, was  $1.9 \pm 0.9\%$ , with *trans* 18:1 being the most prevalent isomer (86%), followed by *trans* 18:2 (11%) and *trans* 16:1 (3%). *Trans* 18:3 isomers were undetectable. The major *trans* 18:1 isomer was  $\Delta^{11}$ trans, as in ruminant fats. Concerning cord plasma lipids (n=31), the TFA level was similar as that found in maternal plasma lipids ( $0.63 \pm 0.14\%$  versus  $0.85 \pm 0.23\%$  of total fatty acids respectively) thus confirming their placental passage. However, the *trans* isomer pattern in cord plasma lipids was different from the maternal's one, especially regarding *trans* isomers of linoleic acid (9*c*,12*c*-18:2). On average, the cord plasma contained half as much of the 9*t*,12*t* / 9*c*,13*t* isomer mix ( $p < 0.001$ ), but 3-times more of the 9*t*,12*c* isomer ( $p < 0.01$ ) than the maternal plasma. In umbilical vein and arteries, TFA were equivalent to 0.4% of total fatty acids, or twice less than in plasma total lipids. Moreover, the *trans* 18:2 isomer level in the phospholipids of the umbilical arterial wall showed a significant positive relation to the 20:3 (n-9) acid level, which is a good EFA deficiency indicator. Nevertheless, for this French population, there was no relation between birth weight or head circumference of the newborn infants and the TFA levels in both adipose tissue and plasma lipids of their mothers.

## Antioxidative Effects of Tocopherols and Tocotrienols in coconut fat related to temperature

Wagner Karl-Heinz, Wotruba Florian, Elmadfa Ibrahim

Institute of Nutritional Sciences, University of Vienna, Austria

Introduction: Tocochromanols are the most important antioxidants in food systems, their effectiveness in preventing lipid oxidation highly depends on the matrix and applied mainly the temperature. This study was aimed at evaluating the free radical scavenging effects of tocopherols and tocotrienols at frying conditions and at ambient temperature.

Methods: Frying conditions were simulated by using a Metrohm Rancimat Model 679.  $\alpha$ ,  $\gamma$ ,  $\delta$ - tocopherols (0.01–0.5%) and  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ - tocotrienols (0.01-0.1) were added to coconut fat. The system was and oxidised in duplicate at 160°C without having an air flow. The development of peroxide value (POV) and conjugated dienes (CD), as the main oxidation products was observed after addition of 0.01, 0.1 and 0.2 % of different tocotrienols to coconut fat, test temperature was 60°C.

Results:  $\delta$ -T was the best protector against lipid peroxidation at frying temperatures. The Induction period (IP) with 0.5%  $\delta$ -T was 14.8 h, IP of the control only 2.1 h. All added tocopherols, except 0.01%  $\alpha$ -T, increased the IP significantly. Similar results, although less pronounced were observed for tocotrienols. At room temperature effectiveness of tocotrienols was  $\delta$ -T3> $\gamma$ -T3> $\beta$ -T3> $\alpha$ -T3 ( $p<0.001$ ). The results were depending on the added amount (% w/w) 0.2>0.1>0.01 ( $p<0.001$ ).

Conclusion: Irrespective of the temperature employed, the protective effects of tocochromanols was dose dependent (% w/w): at 60°C 0.2>0.1>0.01 (tocotrienols) and at 160°C and 0.5 >0.2>0.1>0.01 (tocopherols). Among the tested antioxidants  $\delta$ -tocopherol and  $\delta$ -tocotrienol were found to be most efficient against lipid oxidation considering oxidation temperature. This study shows, that tocotrienols, similar to tocopherols, are good food antioxidants to enhance shelf life of coconut fat both at frying and at room temperature.

**Monday, October 9, 2000, 15:00 (Human Nutrition and Animal Feeding)**

## **$\beta$ -Carotene in Lipid Oxidation**

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The oxidation kinetics of sunflower oil (SO), as well as of pure triacylglycerols of sunflower oil (TGSO) in the presence of different concentrations (0.001 - 0.02 %)  $\beta$ -carotene was studied. The process was performed at high (kinetic regime) and low (diffusion regime) oxygen concentrations at room temperature in the dark and under daylight. The results from the oxidation of SO and TGSO at 100°C in the presence of  $\beta$ -carotene were also presented. It was established that in the antioxidant-free lipid system  $\beta$ -carotene did not give any antioxidative protection. It worked as a prooxidant during the oxidation at room temperature and at sufficiently high oxygen concentration, the effect being more pronounced in the dark than under daylight.  $\beta$ -Carotene increased the stability of tocopherol - containing SO during its oxidation at room temperature and under daylight. This effect is more strongly expressed in a kinetic regime of oxidation. The synergism of  $\beta$ -carotene with the tocopherols was characterized by the stabilization factor F and the activity A. In the kinetic regime of oxidation F and A varied in the interval  $F = 2.0 - 6.3$ , and  $A = 2.7 - 21.0$ . In the diffusion regime  $F = 1.3 - 1.5$ , and  $A = 1.5 - 2.8$ .

**Monday, October 9, 2000, 13:30 (Biochemistry and Bioengineering)**

**Function and evolution of the P450-dependent  
FA hydroxylases**

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France

The  $\omega$ -hydroxylation of fatty acids has been known for over 60 years, when Verkade suggested that end of chain hydroxylation was the first step in converting fatty acids into dicarboxylic acids, leading to their catabolism. In plants, in addition to fatty acid catabolism, the most obvious role of  $\omega$ -hydroxylation is in the biosynthesis of the cuticle, which covers all parts of plants and constitutes the first barrier protecting plants against viral, microbial and fungal pathogens, phytophagous animals, the penetration of pesticides and environmental pollutants, and also against water loss. Hydroxylation is also thought to be a key reaction in the synthesis of oxylipin derived signaling molecules.

In contrast to in-chain hydroxylation which can't be catalyzed by the lipoxygenase/epoxidase pathway or by desaturase-like hydroxylases, hydroxylation at the terminal methyl group, which is thermodynamically unfavored over oxidation at internal positions, appears to be exclusively catalyzed by cytochrome P450 in eukaryotes. Recently, we have cloned and functionally characterized the first plant  $\omega$  and ( $\omega$ -1)-hydroxylases from *Arabidopsis thaliana*, *Vicia sativa* and *Nicotiana tabacum*. While some isoforms exhibit a broad range of substrates (from C10:0 to C18:3), others appear committed to the oxidation of short or medium chain fatty acids. Certain isoforms are highly and selectively induced by infection, jasmonate or peroxisome proliferators. Phylogenetic studies indicate that the plant fatty acid hydroxylases define a new branch of the general P450 tree and share common ancestors with yeast and animal  $\omega$ -hydroxylases.



**Monday, October 9, 2000, 14:00 (Biochemistry and Bioengineering)**

## **Application of lipoxygenases for the preparation of oxygenated lipids**

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Lipoxygenases (LOXs) and other LOX pathway enzymes are potentially able to form a large set of compounds being of commercial interest. Among them are conjugated dienic acids, jasmonates, and volatile aldehydes. Additionally, fatty acid hydroperoxides, formed by LOX, can serve as precursors for further transformation by either enzymes of the so-called LOX-pathway or by chemical reactions. In the case of linoleic acid more than one-hundred products generated from its LOX-derived fatty acid hydroperoxides have been described. Over the last years multiple LOX sequence alignments and structural modeling of the enzyme/substrate interactions were carried out by several groups in order to identify structurally important amino acids within the active site of the enzyme. We showed, that the replacement of one of these amino acids altered the positional specificity of a plant linoleate-13-LOX in favor of 9-lipoxygenation and indicated that the complete conversion of a linoleate-13-LOX to a 9-lipoxygenating species by a single point mutation is possible. In addition, a 9-LOX was converted into a 13-LOX by multiple amino acid replacements. Moreover, the generation of two LOXs with new positional specificities, a linolenate-6-LOX and a arachidonate-11-LOX, was achieved. In addition, applications will be described for a trilinoleate-13-LOX which is capable of oxygenating esterified polyenoic fatty acids such as triacylglycerols. In addition, this enzyme forms with arachidonic acid as substrate, preferentially either 8- or 11-hydroperoxy eicosatetraenoic acid, which is a very unusual positional specificity for plant LOXs.

Monday, October 9, 2000, 14:30 (Biochemistry and Bioengineering)

## **$\alpha$ -Oxidation of Fatty Acids in Plants: Biochemical Fundamentals and Biocatalysis of Enantiomerically Pure (*R*)-2-Hydroxy Acids**

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C.R. Saha-Möller<sup>3</sup>, W. Adam<sup>3</sup>, P. Schreier<sup>1</sup>

Contribution from the Institutes of Food Chemistry<sup>1</sup>, Biochemistry<sup>2</sup>, and Organic Chemistry<sup>3</sup>, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany

The substrate selectivities of the  $\alpha$ -oxidation of saturated, unsaturated, and heteroatom-containing carboxylic acids by an enzyme extract from germinating pea (*Pisum sativum* L.) indicated high enantioselectivity. Synthesis of optically pure (*R*)-2-hydroxy acids was achieved on the semipreparative scale by  $\alpha$ -hydroxylation of long-chain carboxylic acids with molecular oxygen, catalyzed by the pea enzyme. The enzyme was purified by a five-step procedure to apparent homogeneity. The purified protein was found to be a 230 kD oligomer with two dominant subunits, i.e. a 50-kD subunit with NAD<sup>+</sup> oxidoreductase activity and a 70-kD subunit, homologue to a 'pathogen-induced oxygenase' (PIOX), which in turn shows significant homology to animal cyclooxygenase. On-line liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) revealed rapid  $\alpha$ -oxidation of palmitic acid incubated at 0°C with the purified  $\alpha$ -oxidation enzyme, leading to (*R*)-2-hydroperoxy-palmitic acid as the major product together with (*R*)-2-hydroxypalmitic acid, 1-pentadecanal, and pentadecanoic acid. Inherent peroxidase activity of the 70-kD fraction decreased the amount of the (*R*)-2-hydroperoxy product rapidly and increased the level of (*R*)-2-hydroxypalmitic acid. Incubations at room temperature accelerated the decline towards the chain-shortened aldehyde. With the identification of the dual function  $\alpha$ -dioxygenase-peroxidase (70-kD unit) and the related NAD<sup>+</sup> oxidoreductase (50-kD unit) we provided novel data to rationalize all steps of the classical scheme of  $\alpha$ -oxidation in plants.

**Monday, October 9, 2000, 15:00 (Biochemistry and Bioengineering)**

**Studies on the Isolation of 18-Methyleicosanoic Acid containing  
Proteolipids in the Cell Membrane Complex of Keratin Fibres**

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Keratin fibres like wool and human hair mainly consist of two types of cells: cortical cells, which make up the bulk of the fibre, and cuticle cells, which form the outer sheath around the cortex. The cells are separated by a modified cell membrane, the so called cell membrane complex (CMC), which surrounds the individual cells and is the only continuous structure in the fibre. Each cuticle cell is surrounded by a thin hydrophobic membrane called the epicuticle.

The cell membrane domains contain a variety of lipids readily soluble in organic solvents. In analogy to other keratinized tissues like human stratum corneum, these internal lipids mainly consist of cholesterol, free fatty acids, sphingolipids and cholesterol sulfate and probably are organized in bilayer structures. Stearic, palmitic and oleic acid are the main fatty acids of the solvent soluble lipids. The epicuticle, however, has been shown to contain fatty acids covalently bound to the fibre surface proteins. This so called f-layer is responsible for the hydrophobic nature of the surface of keratin fibres.

The main fatty acid of the f-layer is 18-methyleicosanoic acid (18-MEA). In this paper we report on our studies concerning the isolation of 18-MEA containing proteolipid fractions from the fibre, their purification by counter current chromatography and the characterization of their fatty acid and amino acid compositions. Furthermore, the nature of the fatty acid bond to the fibre proteins and the location of 18-MEA proteolipids in the fibre were studied.

Monday, October 9, 2000, 13:30 (Analytical Methods and Management)

## In vitro test for the effectiveness of antioxidants as radical scavengers for thiyl radicals

Erika Klein and Nikolaus Weber

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Free radicals are highly reactive species of chemical compounds which are induced by the transfer of energy, e.g. in the form of irradiation. They are also formed under physiological conditions, particularly by mitochondrial oxidation processes or hepatic cytochrome P<sub>450</sub> redox system. Once formed free radicals attack other cell constituents which are converted to various chemical derivatives. There is increasing evidence that reactions of free radicals and their derivatives are involved in pathological processes leading to several types of human diseases including atherosclerosis, diabetes and cancer. For example, oxidation of low density lipoproteins (LDL) may play an important role in the initiation and progression of atherosclerosis and physiological thiol compounds such as homocystein may play an important role in this mechanism. Various chemical properties seem to be involved in the effects of thiol groups on vascular tissues, predominantly redox potential and capacity for the formation of reactive thiyl radicals (RS<sup>•</sup>).

Attacks of thiyl radicals on the double bonds of unsaturated fatty acids lead to stereomutation (*cis-trans*-isomerization) and addition reactions, i.e. thioether formation. On the basis of these findings an *in vitro* test system has been developed which allows to study the effectiveness of antioxidants in preventing the attacks of free thiyl radicals on unsaturated fatty acids. Various antioxidants were tested for their ability to protect the >C=C< double bond of oleic acid against attacks of thiyl radicals generated from 1-tetradecanethiol by heating. The formation of the *trans*-isomer, i.e. elaidic acid, as well as of the addition products, i.e. isomeric 9(10)-*S*-tetradecyl stearic acids, was determined by gas chromatography as a function of antioxidant concentration. The results show that ascorbic acid 6-*O*-palmitate, octyl gallate and ubiquinone 50 (coenzyme Q<sub>10</sub>) were highly effective in preventing reactions of free thiyl radicals with oleic acid, whereas 2,6-di-*t*-butyl-4-methylphenol (BHT) and *rac*- $\alpha$ -tocopherol were rather moderately active.

**Monday, October 9, 2000, 14:00 (Analytical Methods and Management)**

## **Focus on Iron/Ascorbic Acid induced Oxidation and Antioxidation of Linoleic Acid emulsified in Water**

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The peroxidation of linoleic acid was induced by the Fe<sup>2+</sup>/ascorbic acid system in a partitioned medium and the kinetics of the produced conjugated dienes were followed with and without antioxidants. The originality of our test consists in using a very weak level of ascorbic acid, in order to avoid a superimposed antioxidant activity hindering the mechanistic studies. Moreover, in our conditions (17 μM Fe<sup>2+</sup> and 0.73 μM ascorbic acid) only 5 % of the Fe<sup>3+</sup> species produced during the initiation phase may be regenerated. Other conditions of medium were established to obtain an exponential like profile. With a linoleic acid/Fe<sup>2+</sup> ratio of 10, a steady-state propagation rate is reached after one hour and lasts for up to fifteen hours. Two quantitative parameters were retained for their good reproducibility : first, the slope of the linear part of the propagation kinetics, and second, the extrapolated absorbance of this step at zero time. These two parameters were used in order to compare the antioxidant power of different phenolic compounds.

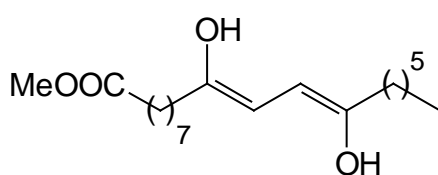
The 17 antioxidants tested cannot avoid the early dienes (30-40 % of the total dienes) resulting from the inducing reactions, whereas they can stop all the dienes produced during propagation reactions by acting on ROO°. The inhibition values reveal great differences between the antioxidants, depending on their structure and on their polarity, confirming the « polar paradox ». Thus, α-tocopherol, BHT, BHA, and eugenol appeared the best, but rosmarinic and caffeic acids, generally potent antioxidants, presented a weak efficiency. Surprisingly, in such a metal induced system, the chelator activity seems to play a minor role.

### Antioxidants based on fatty acids

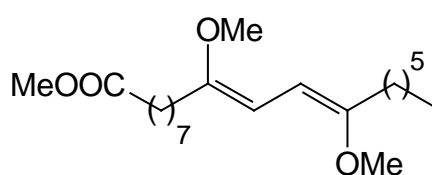
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Autoxidation is the cause for deterioration of commercial organic materials, e.g. plastics, lubricants, fuel and food. Antioxidants<sup>1</sup> are among other measures used to retard unwanted autoxidation. Combination of antioxidants with amphiphilic fatty acids could possibly increase their activity and scope of application. Such conjugates would be also biodegradable and nontoxic.

We succeeded in the synthesis of fatty acid derivatives (**1**, **2**) with an endiol structure like in ascorbic acid. Antioxidants are in general good reducing agents, that means they have low oxidation potentials. In this respect **1** and **2** are oxidised at similar potentials as ascorbic acid.

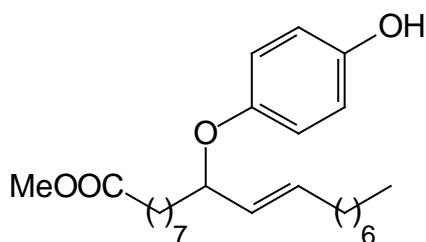


**1**

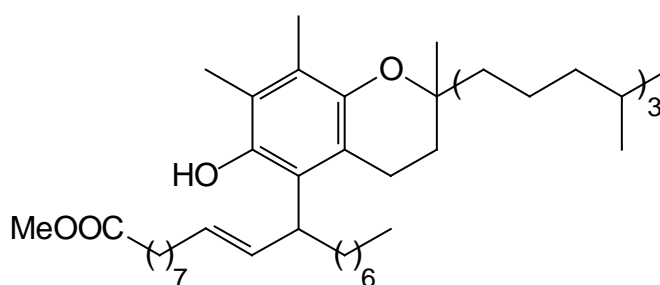


**2**

Other antioxidants like hydroquinone or tocopherol<sup>2</sup> were linked with oleic acid (**3**, **4**).



**3**



**4**

The synthesis and properties of those antioxidants are reported and discussed.

<sup>1</sup> P. P. Klemchuk, *Ullmann* (5.), **A3**, 91-111.

<sup>2</sup> G. W. Burton, K. U. Ingold, *Acc. Chem. Res.* **1986**, *19*, 194-201

**Monday, October 9, 2000, 15:00 (Analytical Methods and Management)**

## **Antioxidant and radical scavenging properties of extracts from different oilseeds**

Bertrand Matthäus, Institut für Chemie und Physik der Fette der Bundesanstalt für Getreide-, Kartoffel- und Fettforschung im Forschungsverbund Produkt- und Ernährungsforschung; Postfach 1705, D-48006 Münster

The autoxidation of lipids is the major reaction in fats, oils and fat containing foods responsible for the deterioration during storage and processing. Antioxidants retard this reaction and the development of unpleasant sensory impressions. Despite the mainly use of synthetic antioxidants there is a general trend and a rising interest in natural antioxidants, whereas tocopherols are the most widely applied antioxidants of this group. Besides, extracts of rosemary or sage, but also of other herbs and spices are good sources for antioxidants. These extracts are mixtures of several compounds with antioxidative effects, especially phenolic compounds such as phenolic acids.

For an added value of the residues of oilseeds obtained during the oil pressing process it could be interesting to use extracts of these residues as natural antioxidants. Therefore it is important to characterize the extracts regarding its antioxidative activity and the composition of phenolic compounds.

After removal of the oil, the residues of fourteen different oilseeds were extracted with a methanol/water mixture for the extraction of the phenolic compounds. The content of phenolic acids and condensed tannins was determined with spectro-photometrical methods and the determination of sinapine and individual phenolic compounds was achieved by HPLC.

The characterization of the extracts with regard to the kinetic behaviour as free radical scavengers was carried out by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as source for free radicals. Further investigations of the antioxidative activity of the different extracts were studied by using luminol induced chemiluminescence as well as evaluation of free radical scavenging activities by electron spin resonance (ESR). The application and the effect of the extracts was also examined by using the Rancimat method with refined sunflower oil. The results of the different methods were compared with results of synthetic and natural antioxidants as well as different phenolic compounds.

**Tuesday, October 10, 2000, 8:30 (Award Lecture)**

**Lipases : Interfacial Enzymes with Attractive Applications.**

Robert Verger *et al.*

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Lipases not only hydrolyze fat in the digestion tract or interesterify triglycerides on a technical scale, but are surprisingly flexible biocatalysts for the acylation or deacylation of a wide range of unnatural substrates. Lipase-catalyzed transformations of racemic and prostereogenic compounds usually proceed with high enantioselectivity. If several functional groups amenable to lipase catalysis are present, the reaction is mostly regioselective. Lipases are used industrially as detergent enzymes, in paper and food technology, in the preparation of specialty fats, and as biocatalysts for the synthesis of organic intermediates.

Lipases were previously defined in kinetic terms, based on “interfacial activation”. This phenomenon was not found among esterases. From an enzymological point of view, some lipases exhibit a unique tertiary structure that exposes the catalytically active site only in the presence of a lipid phase or, presumably, in an organic solvent. Recently determined 3D structures of some, but not all, lipases show a “lid” controlling access to the active site. Thus, the presence of a lid, and “interfacial activation”, are unsuitable criteria for classifying specific esterases. Consequently, lipases can be pragmatically redefined as carboxyl-esterases acting on long-chain acylglycerols.

**Schmid R. D. and Verger R.** (1998). Lipases: interfacial enzymes with attractive applications. *Angewandte Chemie* **37**: 1608-1633.



**Tuesday, October 10, 2000, 9:15 (Award Lecture)**

## **Fatty acids regulate fatty acid metabolism – a nutritional opportunity**

Friedrich Spener, Department of Biochemistry, University of Münster, Germany

The role of dietary fats and oils in human nutrition is one of the most important areas of concern and investigation in the field of nutritional science. New evidence concerning the benefits and risks associated with particular aspects of dietary fat is constantly emerging. On the one hand adequate amounts of dietary fat are essential for health, because in addition to their contribution to meeting energy needs, intakes of dietary fat must be sufficient to meet requirements for essential fatty acids and fat soluble vitamins. On the other hand, excessive dietary fat intake has been linked to increased risk of dyslipidemias, obesity and coronary heart disease. The nutritional value of fat has been traditionally evaluated by epidemiological studies, however during the last years it has become evident that dietary fatty acids affect lipid homeostasis at the genetic level by directly modulating nuclear receptor activity. As a result, new concepts for chips assessing the efficacy of nutrients, including dietary fats and oils, are constantly emerging. One prominent example for fatty acid modulated gene regulation are the isoforms of peroxisome proliferator activated receptor (PPAR) which are activated in particular by agonists of the polyunsaturated and branched-chain type fatty acids and which regulate mitochondrial and peroxisomal degradation of fatty acids. A further class of proteins involved in the signal transduction pathway of fatty acids are fatty acid binding proteins (FABP) which can act as cytosolic discriminatory protein for PPAR agonists by shuttling them to the nucleus for receptor activation. Thus nuclear receptors and fatty acid binding proteins can be future targets for nutritional and biomedical intervention strategies to affect lipid metabolism at the genetic level.

**Tuesday, October 10, 2000, 10:30 (New Generation of Products)**

**NAPUS 2000: Breeding of oilseed rape (*Brassica napus*) for improved human nutrition**

Martin Frauen\* & Wolfgang Friedt\*\*

\*Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, Germany

\*\*Justus-Liebig-Universität Giessen, Germany

Following a competition announcement of the German Ministry of Education and Research (BMBF) a project dealing with the improvement of the nutritional value of oilseed rape (*Brassica napus*) for human food applications was worked out.

A number of 17 partners from different working areas are carrying out a complex project reaching from the discovery, characterisation, isolation and transfer of genes of interest up to breeding of well performing varieties combined with important agronomic traits to economic studies and processing trials as well as nutritional investigations of the new qualities. The partners came together from universities and the business world to undertake a comprehensive research with a high economic perspective.

The project focuses on different seed components which are targets for conventional and biotechnology-assisted breeding strategies. The naturally occurring substances are tocopherols, proteins and lecithins, new substances are the antioxidant resveratrol and long-chain polyunsaturated fatty acids (LCPUFAs) which altogether will result in completely new properties in oilseed rape. Resveratrol is a substance naturally found in the skin of grapes (*Vitis vinifera* L.) acting as an antifungal factor. In mammalian nutrition it is an antioxidant and a moderate consumption has a potential positive influence on cardio vascular diseases. The second novel trait to be introduced into *B. napus* are the LCPUFAs usually found in sea fish oils. These display an important compound of the human diet which is normally non-sufficiently supplied and an improved food intake can significantly reduce heart diseases. A number of different organisms and genes are in work to introduce these special fatty acids in new transgenic rapeseed oil.

Tocopherols, e.g. vitamin E, are natural compounds of oilseed rape being found in a great variation with regard to quality, quantity and ratio. Breeding materials of oilseed rape are screened and selected for the improvement of this trait and are going to be

transformed with genes identified to have an important influence on tocopherol biosynthesis in the plant. Similar approaches are undertaken in the field of lecithin where the first genes are already introduced into oilseed rape. The protein of natural rapeseed is known to be of a high nutritional value. But it is not used in human nutrition due to antinutritional factors like sinapine and tannins in the seed complicating the extraction of functional proteins. Therefore, a low-sinapine rapeseed will be developed. For the extraction of these rapeseed compounds a new technology, the Friolet technique, will be used to guarantee a careful and safe fractionation. All improved or new seed components will be investigated very carefully from the food industry and from food scientists in order to produce novel health food from these new rapeseed qualities.

**Tuesday, October 10, 2000, 11:00 (New Generation of Products)**

### **Aqueous-enzymatic pre-treatment of soybeans**

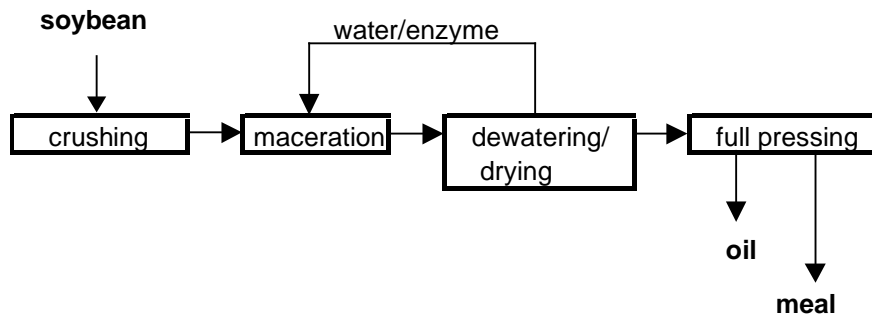
Dr. J. Hollien, Dipl.-Ing. G. Börner, ÖHMI Engineering GmbH (Magdeburg)

Prior art of oilseed processing is the solvent extraction with a high oil yield and rarely full pressing with the disadvantage of high content of residual oil in the cake.

The aim for the research work by ÖHMI was the investigation of alternative processes, that can remove the disadvantages of solvent extraction and full pressing.

The results of previous realised research work can summarised in following:

- A process was developed for aqueous enzymatic treatment of soybeans, that characterised through following process steps:
- With this process on a small technical scale were reached oil yields up to



about 90 % related to the absolute oil content of soybean.

- The aqueous-enzymatic pre-treatment was optimised, so that none significant costs result from enzymes.
- The characteristics of crude oil correspond to the characteristics of conventional produced soya oil.
- The characteristics of press cake correspond to the characteristics of conventional produced soya meal, but there's no trace of solvent.

It will be given an outlook on the economy. From the studies for economy results further need of research work with regard to optimisation of enzymatic maceration and improvement on efficiency of pressing.

This process is economical for oil yield of 95% and an interesting alternative for middle processing capacity.

**Tuesday, October 10, 2000, 11:30 (New Generation of Products)**

### **Deacidification of Fats and Oils by Extraction**

## **with Aqueous Solutions of Amines**

Martin Drescher, Siegfried Peter, Wolfgang König, Eckhard Weidner

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Free fatty acids impair storage life, smell, taste, and appearance of natural fats and oils. After removing finely dispersed solids and phosphorous compounds by degumming and filtration, deacidification is the next step in the refining process. Usually the deacidification is carried out by extracting the free fatty acids by aqueous alkali solutions. At high concentrations of the alkali salts of the fatty acids gelatinous soap stocks are formed. Therefore the deacidification of oils with a high content of free fatty acids is performed by steam stripping at 220<sup>0</sup> to 260<sup>0</sup>C. However, the deacidification by this method results in free fatty acid purities of 0.2 % only. The deacidification of palm oil was investigated by the use of aqueous solutions of amines - especially tertiary amines - as an extractant. Amazingly aqueous solutions of amines do not show the formation of gelatinous soap stocks, when the amine concentration exceeds values of about 20 wt.% . At amine concentrations of the aqueous solutions between about 20 % and 80 %, two phase systems are formed with the oils containing up to 70 % free fatty acids. The resulting two-phase systems contain coexistent liquid phases. Tertiary amines with boiling points ranging between 80<sup>0</sup> and 170<sup>0</sup> C, such as dimethylamino-ethanol, 2-methylamino-diethanol, 4-methylmorpholin, 1-dimethylamino-2-propanol etc., are suitable substances for the deacidification of fats and oils containing free fatty acids under the conditions mentioned. The extract consisting of water, amine, and fatty acids is separated by distillation so as to yield fatty acids and solvent. Purification of the deacidified oil is performed by steam stripping or by washing with water and/or aqueous solutions of volatile acids. The residual concentration of free fatty acids in the refined product is below 0.01 %. The loss of neutral fat during the deacidification process as described is below 1 %.

**Tuesday, October 10, 2000, 10:30 (Human Nutrition and Animal Feeding)**

### **Concept for improving the product safety**

Eberhard Heidenreich, IFF-Research Institute of Feed Technology,

For more than five years the feed mills concentrate their long-time activities of quality assurance on the elaboration, installation, documentation and certification of an effective quality management system according to ISO 9000 et seq. or /and Netherlands System of Good Manufacturing Practice (GMP). At present, it can be said, more than 80% of the industrially produced compound feed comes from certificated feed mills. The principle of these quality assurance systems is the assurance of the product quality by assurance of the required process quality, including all quality relevant processes of the company, i.e. not the production processes only. The successful certification expresses the quality ability of the company and it does not give any confirmation of the product quality. Neglecting the objective description of the quality criterions for compound feed the product quality is interpreted by the guarantee that the product conforms to customer and legal requirements.

Nearly independent of this level of quality management the feed mills in Europe were confronted with some scandals, which make unsure the final consumer concerning the food of animal origin and cause great pressure on feed mills. The producer responsibility for safe food is out of discussion, it is empirically developed and can be found in many legal regulations. Obviously it is difficult to understand that there is no such thing as totally safe food without any risk and own responsibility. Therefore, the discussion about the guarantee of the product safety is directed to the further improvement of the food safety and avoidance of scandals connected with unnecessary uncertainty of the consumer.

The manufacture of compound feed is one part of the production chain for food of animal origin and the slogan "safe feed - safe food" expresses the responsibility of the feed miller for the safe food. But the inversion of this slogan that all defects at safe food of animal origin are mainly caused by unsafe feed is false and it avoids effective concepts for improving the product safety and the food safety respectively.

The lecture demonstrates a possible concept for improving the product safety which uses the hazard analysis and evaluation as starting point and has to be installed along the production chain for food of animal origin. The basis is the method of HACCP,

which more and more is introduced in companies of food manufacturing. For feed mills exist good chances for integration of HACCP into the quality management system. The results of the first hazard analysis identify the interface between the feed mill and the supplier of raw components as well as the carry over of critical additives delivering cross contamination and the infections by pathogenic germs as critical points for manufacturing compound feed. Proposals for monitoring and control of the critical points are essential facts of the concept. Because the critical points become more critical the closer the product gets to the consumer, the effectiveness of the concept will be poor, if no general installation for all parts of the production chain for food of animal origin will be carried out. The definition and control of the interfaces are serious tasks for the realisation of the concept.

**Tuesday, October 10, 2000, 11:00 (Human Nutrition and Animal Feeding)**

### **Effects of crushing conditions on meal quality**

Jacques Evrard<sup>1</sup>, Anne Bourdillon<sup>2</sup>, Pierre Burghart<sup>1</sup>, Marie-Pierre Le Guen<sup>3</sup>

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Four different oil-extraction processes have been applied to a batch of double-low rapeseeds, in the pilot plant of CETIOM. The conditions has been chosen with the aim of protecting the proteins from ruminal degradation. They involved cooking temperature (90°C or 130°C) and desolventation parameters (temperature and steam output). Furthermore, six regular rapeseed meals have been collected on feed market.

On these total of 10 rapeseed meals, classical chemical analysis have been done, as well as determinations of in sacco degradation of dry matter and nitrogen in cows.

The protein degradation in the rumen was decreased to 36% by some of the process conditions, while keeping a good digestibility in the small intestine. The protein value of these pilot rapeseed meals for ruminants was increased by up to 46%. However they were not interesting for poultry feed as the digestibility of some amino acids measured in cockerels was reduced.

As for the regular rapeseed meals, their protein values for ruminants showed a large variation : the N degradatibility in the rumen ranged from 58% to 69%, while the N intestinal digestibility ranged from 66 to 80%.

A similar study was lead with sunflower seeds. Furthermore, 10 regular sunmeals have been collected on feed market.

In comparison with industrial sunmeals, the protein degradation in the rumen was decreased to 38% by some of the pilot process conditions, while keeping a good digestibility in the small intestine. The protein value (PDIE) of the pilot sunmeals for ruminants was increased by up to 68%.

This work has been carried out by EURETEC II (France) which is a collaboration between feeding industry (Ets ARRIVE, CCPA, GUYOMARCH Nutrition Animale, SANDERS, UCANOR, UNICOPA) and PROLEA organizations (CETIOM, ITCF, ONIDOL, SOFIPROTEOL, UNIP), in the framework of the project EUREKA-EUROPROTEINS (EU623), with a financial support of the French Ministry of Research.

**Tuesday, October 10, 2000, 11:30 (Human Nutrition and Animal Feeding)**



## **Improved seed crushing process by means of extrusion**

Pierre Burghart, Jacques Evrard, Hervé Niel, CETIOM, Oilseeds Technology, rue Monge, parc industriel, 33600 Pessac, France.

Some new oilseeds have been identified as potential sources for new industrial applications: fatty acids with specific functionalities, new peptides or enzymes. In many cases, the identified potential markets for these specialty oils or other new products are limited and the seed volumes to process are too low for traditional crushing capacities (1000 to 2000 T per day at minimum). Moreover, development of these new applications need to keep an identity preservation of oil and meal all along the process chain. More generally, utilization of solvent in oilmeal plants could be forbidden in the future for a better respect of environment. In this context, utilization of a twin-screw extruder fitted with a deoiler barrel appears as an interesting alternative technology. This system has been developed by Clextal Company (Firminy, France) in connection with the Technology University of Compiègne (France) and was the subject of a Ph.D in 1994. The technical feasibility of this extruder has been demonstrated on rapeseed and sunflower (dehulled and non-dehulled) and castor. The studies demonstrated the possibility to process dehulled rapeseed meal with 8 to 10% of residual oil. This new process allows a good flexibility : the unit processes of the traditional pressing, which are flaking, cooking and pressing are made by this single machine. This technology should also improve meal and oil quality : The process is done in a time which doesn't exceed 3 to 5 minutes (vs 45 to 60 minutes in the traditional process). The machine can be heated by heating bands, or cooled by water circulation in sleeves. In these conditions, an added value for meal could be obtained by the extrusion technology in preserving the protein functionalities during the oil extraction process and the reduction of non hydratable phospholipids in crude oil should allow the water demucilagination and reduce phosphorus wastes during refining. A pilot extruder (50 to 150 Kg/h) is actually operating by CETIOM, in the CREOL oil mill pilot plant, with a grant of AGRICE (the French Agency for Environment and Energy Management). The research mainly includes the optimization of deoiling with different oilseeds and the examination of oil and meal quality.

**Tuesday, October 10, 2000, 10:30 (Biochemistry and Bioengineering)**

## **Lipase deactivation in oil**

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Over the last two decades the use of enzymes has become an important tool in the food industry, e.g. using lipases for the modification of oils, fats and their derivatives. This at least is the impression which emerges from the vast amounts of literature published on the subject. However, in practice the number of full-scale processes in the food industry based on lipase catalysed conversions is rather small and is predominantly limited to high value products such as cocoabutter equivalents and fats for infant formulas.

Without doubt this situation is partly caused by the relatively high prices of food-grade enzymes, but the re-use of the lipase and an increase of its operational stability are equally important. Immobilisation has widely been investigated to allow easy re-use of the enzymes, but only limited information is available regarding the operational stability of lipases, both free and immobilised.

On the one hand, some researchers have shown the impact of the oil feed quality on the operational stability, i.e. the half-life time, of the biocatalyst. The results reported suggest this oil quality is linked to its state of oxidation, but further details are not available. On the other hand, medical research into the mechanisms of lipid oxidation in lipoproteins have shown many physical interactions and chemical reactions to take place between lipid oxidation products and proteins.

Despite all this knowledge, a large gap does exist towards understanding the loss of enzyme activity during oil modification, let alone solving the issue. Based on a review of existing literature, this paper aims to re-focus attention to this 'knowledge gap' trying to open up the area.

**Tuesday, October 10, 2000, 11:00 (Biochemistry and Bioengineering)**

## **Margarine fats from enzyme technology: a large-scale evaluation**

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Margarine fats are daily-used fats. The market is very large but the price is generally low. There have been discussions whether such products can be produced using enzyme technology in an economically balanced way because enzymes are still relatively expensive and the technology is not used in a routine operation. The advantages using enzyme technology are immense, such as mild conditions, less pollution, green process and products, less process investment, etc. Besides these points, products from enzyme technology may have more nutritional benefits than those from chemical methods with respect to enzyme specificity. If these benefits outweigh the shortcomings of the technology, especially when enzyme price comes further down and enzyme stability is longer, the market for enzymatically produced margarine fats can be changed. In this report, studies have been carried out in large scales and trials in 300 kg level was also conducted. The performance was not only monitored by triacylglycerol profiles, diacylglycerol content, and free fatty acid content, but also by physical properties and enzyme stability. A toolbox for industrial applications is provided.

**Tuesday, October 10, 2000, 11:30 (Biochemistry and Bioengineering)**

## **Lipase-catalyzed synthesis of structured triglycerides**

### **- From basic research to Miniplant production scale -**

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- 3) Institute for Chemistry & Biochemistry, University of Greifswald, Soldmannstr. 16, D-17487 Greifswald, Germany

1,3-Oleoyl-2-palmitoylglycerol (OPO), a structured triglyceride, used as a human milk replacer in infant nutrition, is produced by a one-step lipase-catalyzed process in industry. The aim of a high yield of pure triglyceride with the desired structure motivated the development of a new process based on a lipase-catalyzed two step reaction. In the first step tripalmitin is converted to 2-monopalmitin catalyzed by a sn1,3-regiospecific lipase.

2-monopalmitin is then esterified in a second step with oleic acid to produce OPO using the same enzyme as in step one [1].

A kinetic model describing the synthesis of OPO was developed and the parameters of the model were estimated by kinetic studies. Subsequently, a miniplant, in the sense of a small representation of a imaginable production plant including the various unit operations (fixed bed and membrane reactors, pervaporation moduls) has been designed and implemented. The plant was also equipped with online analytics (gaschromatography) and computer aided control system. The system mimics production conditions as far as substrate concentrations and material flow are concerned. The application of a modular miniplant concept along with mathematical modeling and simulation is an important tool for reducing the time span between basic biological innovation (screening and/or protein engineering) and getting the product to the market.

References:

- [1] U. Schmid, U.T. Bornscheuer, M.M. Soumanou, G.P. McNeil, R.D. Schmid, 1999, Biotechnol. Bioeng. 64, 678-684

**Tuesday, October 10, 2000, 10:30 (Analytical Methods and Management)**

**Determination of unsaponifiable matter  
by fluorescent labeling HPLC**

Dr. Mörsel, Jörg-Thomas, Inst. for Food Chemistry, TU Berlin

The determination of unsaponifiable matter is a general problem in lipid analysis. Commonly the determination is carried out by saponification of glycerides and other esters. In general a pre-concentration in the ratio up to 1:100 is achieved. There are some important reasons against the saponification step, mainly losses in analytes and chemical reactions, e.g. destruction and modification of substances. The direct determination of minor components is often difficult because of low concentration and interference. Today only a few substances can be determined. The labeling of reactive compounds with fluorescent dyes and determination with HPLC – fluorescent detection may be a way for a more pleasant analysis.

We investigated plant oils for the determination of sterols and tocopherols by HPLC with fluorescent detection. Both groups of minor components can be determined without further clean-up steps by reaction with 7-diethylaminocoumarine, 1-pyrenebutyrylacidhydrazide, anthrolynitril, dansylfluoride and other.

Determination was carried out with either normal phase or reversed phase HPLC. RP chromatography seems to be the more powerful method from the view of resolution. Normal phase HPLC is more convenient due to the problems arising from the glyceride matrix. Thus RP chromatography also can be carried out with back flush technique. Because of the low detection limit (in some experiments 10 fg) only small amounts of neutral lipids are given to the columns. Tocopherols as well as sterols are separated quickly and for tocopherols with good resolution. In the case of sterols sometimes overlapping peaks of different sterols may be a problem. The great advantage of fluorescent labeling analysis is the direct determination without any time and cost extensive sample preparation step.

**Tuesday, October 10, 2000, 11:00 (Analytical Methods and Management)**

### **Sterol Analysis by LC-GC**

Ludger Brühl

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The analysis of sterols is a powerful tool for the chemist to identify fats and oils. Vegetable oils show most often a characteristic sterol composition. In many cases blends can be identified. The sterol composition also reflects the processing of an oil. This enables the control of extra virgin olive oils and other native oils labelled as cold pressed.

The analysis according the updated official DGF standard method is very precise and gives a lot of information to the analyst. However, the method is time consuming and skilled staff is needed. In order to facilitate and automate this procedure new methods using LC-GC have been published with different approaches to this field of analysis.

We tested an automated programmed temperature injector for large volumes in combination with a flow cell access for the injection syringe as a possibility to couple LC and GC. The eluate from an HPLC of common dimensions passes after a UV detector a flow cell with a septumless head, which is used in a sample position for an automated large volume injector. This injector is capable to inject volumes of up to 1000 microliters in a programmed temperature injector, where the solvent is vented before the sterols are transferred splitless onto the capillary column.

The sample preparation is very quick. The oils are transesterified within 20 min and injected into the HPLC for analysis. This enables the analysis of total sterols, as common practice with the official DGF standard method. Sterol esters are covered as well as free sterols in order to determine the total sterols as the sum of free and esterified sterols. The laboratory work is cut down from about a half a day to an half an hour.

However, the results obtained with this new method are not as precise as the results obtained with the standard method. Differences in the resolution of the free sterols in the chromatogram as well as problems with the reproducibility of the procedure give rise to some drawbacks for this method.

- 1) Brühl, L. and Fiebig, H.-J., Am. Oil Chem. Soc. **75** (1998) 895.
- 2) Aitzetmüller, K.; Brühl, L. and Fiebig, H. J., Fett/Lipid **100** (1989) 329.
- 3) Biedermann, M.; Grob, K. and Mariani, C, Fat Sci. Technol. **95** (1993) 127.

**Tuesday, October 10, 2000, 11:30 (Analytical Methods and Management)**

## **Determination of Polycyclic Aromatic Hydrocarbons in Lipidic Matrices - Multicartridge Extraction Method**

Dorothee DUBOIS & Renée RAOUX

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The existing method (ISO / NP 15753) for the determination of PAHs in lipidic matrices is time consuming, and not very environmentally acceptable: approximately 800 ml of solvents are used for each extraction; 3 to 4 days are necessary to perform the extractions corresponding to the quantification of a single sample. Consequently, it appears essential to provide a simplification and a miniaturisation of this procedure.

ITERG has already developed micro-methods using commercial cartridges for the determination of pesticides and polychlorobiphenyls (PCBs) in fats and oils (OCL, Vol.4 n°1, p. 71-80, Jan-Feb 97).

Because of the extremely low amounts of PAHs (lower or near to 1 ppb), they should be extracted from a sufficient quantity of oil sample: 2.5 g. This sampling is too important to be purified directly on cartridges; so a preliminary extraction step is necessary.

The main steps of the developed procedure are:

- step 1: extraction of the PAHs from the oil and evaporation of solvents
- step 2: extraction of the PAHs from the extract obtained at step one
- step 3: purification on C18 cartridge and evaporation of solvents
- step 4: purification of the last extract on Florisil cartridge and evaporation of solvents.

The whole quantity of solvents necessary for this procedure is less than 130 ml: one of the reasons is the by-passing of the saponification step described in the ISO method.

The analysis of the extracted PAHs is done by HPLC-fluorimetry.

The method, perfected on sunflower oil, allows obtaining PAH recoveries in agreement with the recommendations of the CEN standards for pesticides and PCBs (70-110 %). The detailed results for each PAH are presented in table 1.

Table 1

Recoveries obtained using ITERG's procedure for refined sunflower oil spiked with 5 µg/kg of each PAH

HAP	NUMBER OF RINGS	MEAN (5 assays)	RSD (%)
Naphthalene (**)	2	-	-
Acenaphthene (**)	3	-	-
Fluorene (**)	3	-	-
Phenanthrene (*)	3	99,3%	19,1%
Anthracene	4	69,8%	14,3%
Fluoranthene	4	89,4%	3,7%
Pyrene	4	87,4%	6,4%
B(a)anthracene	5	86,9%	4,1%
Chrysene	5	89,3%	3,2%
B(b)fluoranthene	5	83,3%	4,4%
B(k)fluoranthene	5	80,4%	4,4%
B(a)pyrene	5	70,0%	5,0%
DB(a,h)anthracene	5	80,6%	2,6%
B(ghi)perylene	6	67,7%	9,8%
I(1,2,3-cd)pyrene	6	75,4%	2,7%

(\*) Phenanthrene: high relative standard deviation (RSD) due to initial presence of the compound in the initial matrix. (\*\*) Naphthalene, acenaphthene & fluorene are volatile PAHs lost during the evaporation steps.

*This work has been supported by ACTIA (Association de Coordination Technique pour l'Industrie Agroalimentaire)*

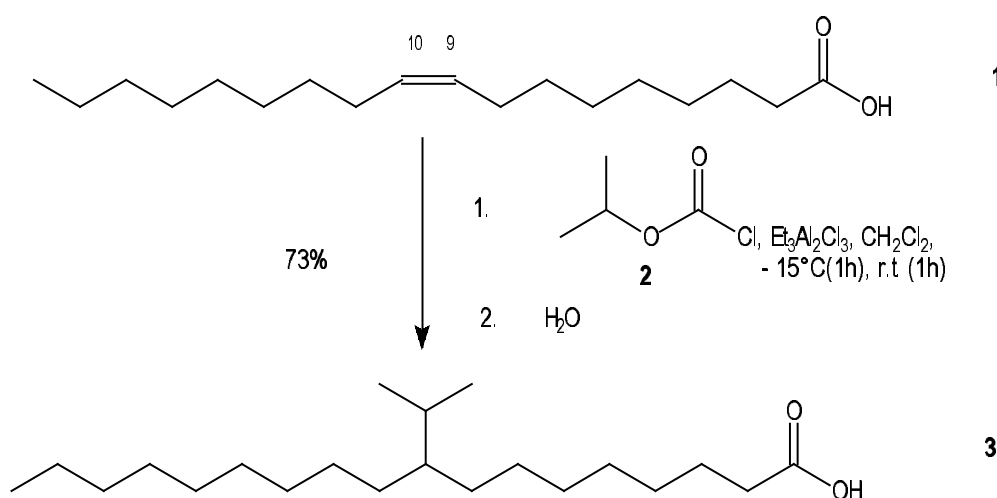


Tuesday, October 10, 2000, 13:30 (New Generation of Products)

## Synthesis of Alkyl Branched Fatty Compounds<sup>1</sup>

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The alkylation of alkenes is a reaction of great importance. We have been interested in alkylation reactions of unsaturated fatty compounds such as oleic acid and native oils that are important renewable raw materials, because branched fatty compounds are known to have interesting properties which are useful in lubricant area and in cosmetic formulations. Methods of the direct alkylation of non activated C,C-double bonds with simple primary and secondary alkyl groups have been unknown. Here we report on Friedel-Crafts alkylations of unsaturated fatty compounds using alkyl chloroformates. The reaction of oleic acid (**1**) with isopropyl chloroformate (**2**) gave in the presence of ethylaluminiumsesquichloride ( $\text{Et}_3\text{Al}_2\text{Cl}_3$ ) after a reaction time of 2 h an approximately 1:1-mixture of the regioisomers 9- and 10-isopropyloctadecanoic acid (**3**) in a yield of 73%.



Obviously the isopropyl cation that is generated from the isopropyl chloroformate in the presence of  $\text{Et}_3\text{Al}_2\text{Cl}_3$  adds to the C,C-double bond of the fatty acid. Transfer of hydride from  $\text{Et}_3\text{Al}_2\text{Cl}_3$  to the adduct carbenium ion gives the saturated product **3**.

The  $\text{Et}_3\text{Al}_2\text{Cl}_3$ -induced alkylation with different alkyl chloroformates such as **2**, isobutyl- and neopentyl chloroformate was applied in addition to ricinoleic acid and 10-undecenoic acid, to the respective methyl esters and to sunflower oil. In some cases addition of a hydride donor such as triethylsilane was necessary.

<sup>1</sup> U. Biermann, J.O. Metzger, *Angew. Chem. Int. Engl.* **1999**, 38, 3675

**Tuesday, October 10, 2000, 14:00 (New Generation of Products)**

**Fats as Raw Materials for Performant Polymers**

J. Bordado, Lisbon, Portugal

Due to a cancellation of the author, this contribution was removed from the program. The following lectures in this session will start 30 min earlier as announced in the program.

**Tuesday, October 10, 2000, 14:00 (New Generation of Products)**

## **Synthesis of Abrasive Soaps with Industrial By-product from Sunflower Oil Dewaxing.**

X.Pages, C.Alfos, K.David and C.Birot  
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Fax : + 33 5 56 36 57 60 – e.mail: [xavier-xatart-pages@wanadoo.fr](mailto:xavier-xatart-pages@wanadoo.fr)

Crude sunflower oil contains natural waxes in variable and small quantities (500-1200 ppm), which are removed at the dewaxing step of the refining ; the dewaxing process is generally performed by oil cooling, maturation at low temperature and separation of the cristallised waxes by centrifugation or filtration.

The use of filter aid (diatomite) is recommanded to improve the filtration rate but leads to the production of a filter cake which is considered as a waste product.

Different samples of industrial filter cakes have been analysed to find some possible ways of valorization. A typical filter cake contains water (10%), sunflower oil (45%), waxes (6 –8 %) and mineral compounds (40 %).

Trials of direct saponification with bases as reactants were conducted both on laboratory and pilot scale ( 5 Kg).

An abrasive soap is obtained according to the semi-boiled process by direct saponification of the oil in the filter cake.

The process is very simple and economical because of no losses accur and no by-products formation during the reaction. Particularly, the glycerol and waxes are remaining in the final product improve its mildness in use.

The obtained soap (called SWS : Sunflower waxy Soap) shows a pasty form and a white colour. It contains about 52 % of water, 20% of soap, 22% of abrasive mineral particles, 3% of waxes and 3 % of non- saponifiable matter.

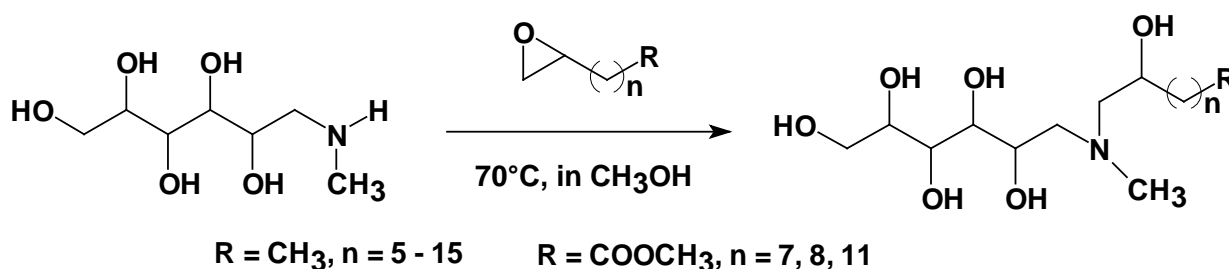
Its use as an abrasive hand soap for the removal of grime, grease and imbedded soil has been studied comparatively to a classical commercial product. Due to its particular composition (waxes, soap content) and the small size distribution of the mineral particles, the Sunflower Waxy Soap shows better cleaning properties and is more mild to the skin.

### Syntheses of Surfactants from Oleochemical Epoxides

S. Warwel, F. Brüse, H. Schier, M. Rüschen, Klaas and B. Wiege  
Institute for Biochemistry and Technology of Lipids, H.P. Kaufmann-Institute,  
Federal Centre for Cereal, Potato and Lipid Research  
Piusallee 68, D-48147 Münster, Germany

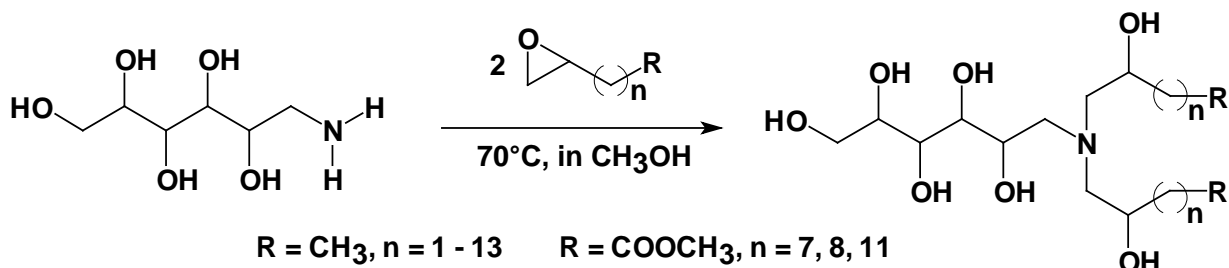
Glucamine and alkylglucamines are industrially available by reductive amination of glucose. Starting with other reducing carbohydrates, further polyolamines are obtainable. By condensation with fatty acid derivatives glucamines are converted to glucamides, which are used in dish washing formulations.

We studied the reaction of epoxides of  $\alpha$ -olefines ( $C_4$ - $C_{18}$ ) with glucamines under mild conditions ( $70^\circ\text{C}$ ) in methanol and the surfactant properties of the according products. Further we investigated the analogous reactions with epoxides of terminal unsaturated fatty acid esters ( $C_{10}$ ;  $C_{11}$ ;  $C_{14}$ ) with polyolamines based on glucose and other reducing carbohydrates. Most of the resulting sugar surfactants were obtained in quantitative yields.



Depending on the chain length of the alkyl group, the used polyolamine the products reduce the surface tension of water down to 25 mN/m at the cmc.

Starting with glucamine (or other primary sugar amines), products with Y-structure were formed, which are surfactants as well as potential monomers for special polyesters, using  $\omega$ -epoxy fatty acid esters for the synthesis.



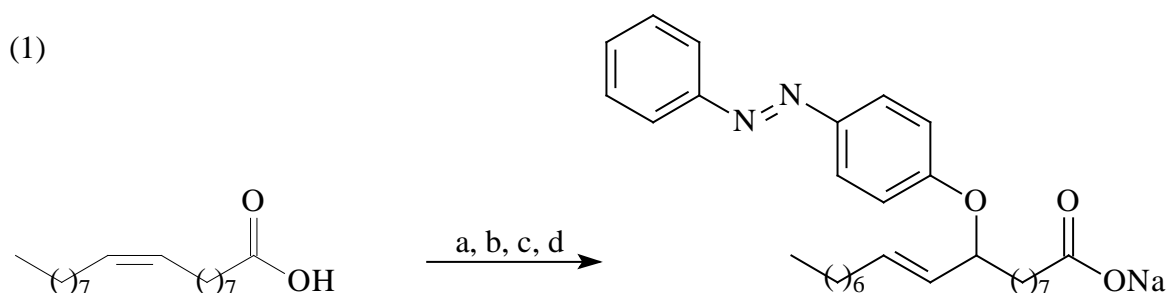
In a following step the reaction products of  $\omega$ -epoxy fatty acid esters were saponificated catalyzed by enzymes or bases to obtain further interesting surfactants (amphoteric detergents).

## Fatty acids linked with dyes and corrosion inhibitors

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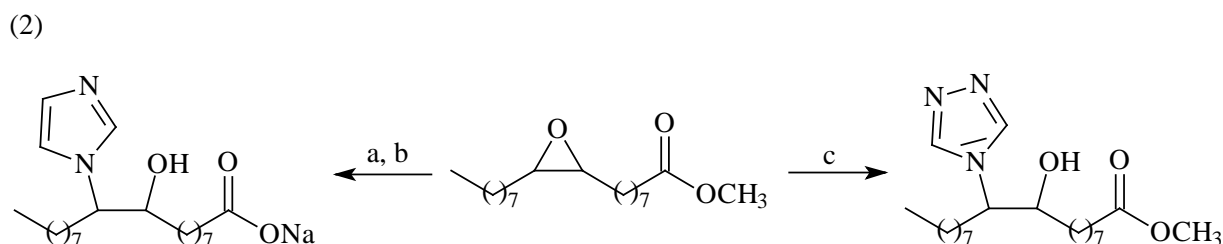
Numerous applications of fatty acids are based on their amphiphilic character. The combination of these amphiphiles with dyes (1) or corrosion inhibitors could lead to new applications and improved physical and chemical properties of these materials.

For that purpose oleic acid was linked with azo compounds by allylic oxidation followed by *Mitsunobu*-reaction (eq. 1).



a) MeOH, DMP, H<sup>⊕</sup>, 95 %; b) <sup>1</sup>O<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, PPh<sub>3</sub>, 98 %;  
c) DEAD, THF, 72 %; d) NaOH, MeOH, H<sub>2</sub>O, 98 %

Corrosion inhibitors were coupled with the fatty acid by nucleophilic opening of the corresponding epoxides.



a) Imidazole, THF (reflux), 93 %; b) NaOH, MeOH, 86 %; c) 4-Aminotriazole, DMSO, 110°C, 60%

These conjugated fatty acid-corrosion inhibitors show interesting retarding effects which were revealed in an electrochemical test (2).

The syntheses and properties of these new fatty acid-conjugates are reported and discussed.

1) J. Heesemann, *J. Am. Chem. Soc.* **1980**, 102, 2167-2181

2) K. Saubier, J. W. Schultze, J. Geke, *Electrochim. Acta* **1994**, 39, 1171-1178.

**Tuesday, October 10, 2000, 13:30 (Human Nutrition and Animal Feeding)**

## **Rapeseed and rapeseed press cake in farm animals affect the quality of milk and meat**

Friedrich Schöne, Gerhard Jahreis, Gerhard Flachowsky, Ulrich Kirchheim  
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In ruminants long chain polyunsaturated fatty acids (PUFA) of rapeseed are modified by rumen biohydrogenation resulting in saturated, but also in transisomeric fatty acids (TFA) transferred to tissues and milk. In pigs glucosinolates may affect growth, i.e. carcass composition. The review is based on experiments with a total of 158 growing pigs, 40 growing bulls and 60 dairy cows receiving high amounts of rapeseed oil directly or via ground rapeseed or high fat rapeseed cake. In a butter market monitoring study 16 samples of a butter produced from milk of cows fed 350 - 400 g/day rapeseed oil equivalents were compared with 16 butter samples from standard segment available on the market. In fattening animals no feeding effects on carcass and meat quality parameters, i.e. pH, meat colour, intramuscular fat content, shear force and sensory characteristics could be detected. Depending on the amount of dietary rapeseed fat, the subcutaneous fat, particularly in pig carcasses, contained more PUFA; for every 10 g rapeseed fat/kg diet there was an 0.8 % increase of linoleic acid and an 0.3 % increase of linolenic acid (C18:3 n3)(basis 100 % fatty acid methyl esters). For human nutrition a certain degree C18:3 n3 in sausages seems to be desired. A higher oxidation susceptibility and lower storability of high PUFA fat was counteracted by tocopherols of rapeseed oil and by dietary tocopheryl acetate supplements. In bulk milk samples rapeseed oil feeding of dairy cows lowered the palmitic acid content and increased the oleic acid content. The softer milkfat and the better spreadable butter contain more TFA. However, conjugated linoleic acid (CLA) represents an important part of TFA. CLA has some beneficial effects in contrast to the most TFA. In pigs as well as in ruminants additional rapeseed fat equivalents should be limited to 20 - 30 g/kg feed dry matter. Pig diets with rape cake do not reach this fat content because glucosinolates limit dietary level of this feed ingredient.

## **Modification of fatty acid composition in organs and egg yolks of laying hens by selenium**

Klaus Schäfer, Institut für Tierernährung der Freien Universität Berlin

Background: The essential fatty acids (EFA) are converted to polyunsaturated fatty acids (PUFA). The content of PUFA, mainly generated through elongation and desaturation from linoleic and alpha-linolenic acids, is influenced by dietary changes. Appreciable evidence exists that the composition of fatty acids in tissues and organs is affected by antioxidant trace elements. In animals in which antioxidant defense mechanisms are depressed, such as in selenium deficiency, long chain PUFA of membrane phospholipids are especially susceptible to lipid peroxidation. The selenoenzyme glutathione peroxidase seems to be one of the most important extra- and intra-cellular antioxidant.

Methods and results: In order to study the role of selenium in affecting the fatty acid composition of eggs as well as laying hens' organs and tissues two dietary fatty acid sources - free linoleic acid as 18 : 2 (n-6) supply and partially hydrogenated soybean oil, rich in monounsaturated trans fatty acids - were fed to laying hens. The fatty acids were fed without selenium supplementation or in combination with selenium supplementation. Dietary treatments had no effect on egg production. The proportions of n-3 PUFA (predominantly DHA) and n-6 PUFA (predominantly AA) in the phospholipids of heart, liver and egg yolk were higher when the diet was supplemented with selenium. However, the effect of selenium was more pronounced in the n-3 group than in the n-6 group. As a consequence of the selenium induced alterations a reduced ratio between total n-6 and n-3 PUFA was observed. In egg yolk the relationship of the incorporation of n-3 PUFA in phosphatidylethanolamines (PE) and in phosphatidylcholines (PC) was influenced by selenium in the linoleic acid group. The preferential incorporation of n-3 PUFA into PE was existent (PE/PC = 3.1) but it was less pronounced when selenium was supplemented (PE/PC = 2.2)

Conclusions: Despite taking into account only analyzing pooled samples of the different dietary groups, these data support the role of selenium as an essential antioxidant which influences the fatty acid composition. Presumably, the beneficial properties of selenium are not due to a single antioxidant effect, but they are produced by additive effects. Further studies with larger samples series will elucidate these aspects and the importance of selenium supplementation.

**Tuesday, October 10, 2000, 14:30 (Human Nutrition and Animal Feeding)**

### **Lecithin in piglets diets – Effect on nutrient digestibility and utilization**

Jensen, S.K., Jørgensen, H., Hedemann, M.S. Isabel, B. & Danielsen, V.

Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology,  
Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, DENMARK

Lecithin commonly refers to a mixture of phospholipids dissolved in oil. Crude lecithin contains approximately 60% phospholipids and 30% oil. Lecithin is an important natural emulsifier in food, feed, pharmaceutical and technical products. The major source of crude lecithin is the degumming step in vegetable oil refinement processing with soybean and rapeseed oil being the major sources.

From the literature there is evidence that phospholipids positively influence the digestive processes of domestic animals, but the mechanism behind the effect remains unclear. Phospholipids are amphiphilic compounds with excellent emulsifying properties. Due to their emulsifying properties they are thought to enhance the effects of the digestive enzymes on the substrate nutrients. In other experiments phospholipids have proved to have a beneficial effect on the action of and perhaps the secretion of various bile acids. Furthermore, they are of importance in promoting fat transport between liver and tissue and in the metabolite exchange between cells. Finally, phospholipids provide an adequate supply of essential building blocks of choline, inositol, ethanol amine, linoleic and linolenic acid.

In the present experiment 54 piglets, weaned at three weeks of age, were used in a digestibility and balance experiment and 180 piglets were used in a production experiment. Nine different diets were manufactured. The diets were based on wheat, barley, fishmeal and dehulled soybean meal. To these basal diets were added either no fat or 50 g/kg of animal fat or rapeseed oil and within these three groups each diet was added either 0, 20 or 40 g/kg of crude lecithin. Both experiments lasted four weeks. In the digestibility and balance experiment three collection periods of faeces and urine were included. At the end of the experiment the piglets from the digestibility and balance experiment were killed. Blood samples were taken immediately, the pancreas and the liver were removed and weighed and a 20 g liver sample was frozen. The whole intestine was removed, the small intestine cut into four sections and the content frozen and stored separately.

Addition of lecithin significantly improved the amount of nitrogen retained within the piglet ( $P < 0.001$ ), the apparent digestibility of tocopherols as well as feed utilisation ( $P < 0.05$ ). Results from the production experiment showed equal performance of the piglets with all dietary levels of lecithin. The feed utilization was also improved in the production experiment with increased dietary level of lecithin. Analyses of the digesta from the four different sections of the small intestine, showed that starch disappeared faster from the first part of the small intestine with increasing lecithin content in the diet, as did the tocopherols.

The activity of the digestive enzymes, trypsin, amylase and carboxyl ester hydrolase in pancreatic tissue and digesta from the four segments of the small intestine was measured. The activities of some of these enzymes varied with fat source and lecithin level. The significance of these variations in enzymatic activity and the measured digestibilities, N- and energy balance may explain some of the beneficial effects of lecithin as a feed ingredient.



**Tuesday, October 10, 2000, 15:00 (Human Nutrition and Animal Feeding)**

### **Effect of PUFA at *sn*-2 Position in Dietary Triglycerides on Fatty Acid Composition of Adipose Tissue in Growing-Finishing Pigs**

M.R.L. Scheeder<sup>1</sup>, D. Gumy<sup>2</sup>, P. Lambelet<sup>2</sup>, C. Wenk<sup>1</sup>, <sup>1</sup>ETH Zurich, CH-8092 Zurich, <sup>2</sup>Nestec Ltd, Nestlé Research Centre, Vers-chez-les-Blanc, CH-1000 Lausanne 26

The fatty acid composition of pig body fat is strongly influenced by dietary fatty acids. Plant oils which are rich in polyunsaturated fatty acids (PUFA) particularly increase the PUFA content in pig fat. One explanation for a very efficient incorporation of PUFAs from plant oils could be the specific composition of their triglycerides with PUFAs predominantly esterified at the *sn*-2 position. As pancreatic and lipoprotein lipases specifically hydrolyse fatty acids at *sn*-1 and *sn*-3 positions, it could be assumed that fatty acids at *sn*-2 position are less affected by metabolic processes and, therefore, more readily incorporated and kept in adipose tissue. A feeding experiment with 48 pigs was conducted to test this hypothesis. The animals were allocated to four experimental groups and fattened from 25 to 105 kg liveweight on a barley/soybean meal diet, supplemented with 4 % of one of four different fats. The added fat consisted of soybean oil (S) and beef tallow (T), mixed in a ratio of 3/1 (S/T) and 1/3 (T/S). Half of each blend was left unmodified (N) or was interesterified (R) using C<sub>2</sub>H<sub>5</sub>Na as catalyst to randomise the fatty acid profile at the *sn*-1,2 and 3 positions. As intended, there was no difference in fatty acid composition between native and randomised batches within one blend: about 26 % or 48 % saturated fatty acids, 27 % or 34 % monounsaturated fatty acids (MUFA), and 48 % or 18 % PUFA in S/T or T/S, respectively. Randomisation, however, markedly altered the proportion of fatty acids at the *sn*-2 position with 60 %, 46 %, 24 % and 17 % PUFA for S/T-N, S/T-R, T/S-N and T/S-R, respectively. Average daily gain and carcass composition was equal for all experimental groups, which indicates that neither the blend ratio nor the randomisation affected fattening performance and muscle or adipose tissue growth. The fatty acid composition of subcutaneous fat (outer layer of backfat) as well as perirenal fat was significantly affected by the ratio of the two fats used, with S/T animals being higher in PUFA mainly at the expense of MUFA. There was, however, virtually no effect of randomisation on fatty acid composition of these adipose tissues (24.6 %, 24.7 %, 17.2 %, 16.9 % PUFA in backfat and 20.9 %, 20.3 %, 13.9 %, 15.0 % PUFA in perirenal fat of S/T-N, S/T-R, T/S-N and T/S-R, respectively). We conclude, therefore, that, despite the specific mode of action of endogenous lipases, the position of PUFA in dietary triglycerides is of minor relevance for the composition of depot fat in growing-finishing pigs.

**Tuesday, October 10, 2000, 13:30 (Biochemistry and Bioengineering)**

## ***Phospholipases D – Structural and application-oriented aspects***

**R. Ulbrich-Hofmann, F. Hirche, I. Schäffner, J. Mansfeld**

*Department of Biochemistry/Biotechnology, Martin-Luther University Halle-Wittenberg, Kurt-Mothes-Str. 3, D-06120 Halle, Germany*

Besides hydrolysis of phospholipids at the terminal phosphate diester bond, phospholipases D (PLD) are able to transfer the phosphatidyl moiety to different types of alcohols. This reaction has been applied in syntheses of manifold phospholipids on laboratory as well as industrial scales, although knowledge on the structural properties of these enzymes is still poor. In the present contribution, PLDs from cabbage and two different *Streptomyces* species, being mostly used for synthetic purposes, are compared with respect to hydrolysis and transphosphatidylation to hydrophilic and amphiphilic primary and secondary alcohols in different solvent systems. The kind of organic solvent used in emulsion systems is shown to influence the total activity as well as the ratio of transphosphatidylation to hydrolysis in the same way for the three PLDs tested. On the other hand, the ratio of transphosphatidylation to hydrolysis is strongly dependent on the nature of the acceptor alcohol and the source of PLD. Hitherto, correlations of experimental results with structural data of PLDs have not been possible. Recent information on gene structures of a great number of enzymes belonging to the so-called PLD superfamily and the first elucidated crystal structure of a related endonuclease promise rapid progress in the recognition of structure-function relationships. New results on the genomic structure and expression of two PLD isoenzymes from cabbage in *E. coli* are reported and discussed in context with available structural data of other plant and microbial PLDs.

**Tuesday, October 10, 2000, 14:00 (Biochemistry and Bioengineering)**

**Using immobilized, phospholipid-modifying enzymes  
in synthesis.**

Peter Grunwald, Department of Physical Chemistry, University of Hamburg,  
Bundesstrasse 45, D-20146 Hamburg.

Phospholipids are used as emulsifying agents and surfactants in food industry, as ingredients in cosmetics, or for the formulation of pharmaceutical products (liposome technology). Some phospholipids as for example phosphatidyl choline (PC) or phosphatidyl serine (PS) are meanwhile known as therapeutically active components. It can be expected that due to the progress in the field of phospholipid synthesis the number of possible applications will increase significantly.

For the modification of (natural) phospholipids hydrolytic enzymes like phospholipase A<sub>1</sub> (PLA<sub>1</sub>), PLA<sub>2</sub>, and PLC, as well as PLD that is unique with respect to its potential to catalyse the polar head group exchange, play an important role (1,2). Furthermore, several lipases with phospholipase A – activity have been found recently. As many of these enzymes are expensive they should be transferred into a re-usable state prior to their application. This can for example be achieved by immobilizing them via covalent attachment to a solid, water-insoluble support. Other advantages of enzyme immobilization are a simple separation of the catalysts from the reaction mixture, and their application in different types of reactors for a continuous and economic production.

The covalent attachment of phospholipases – especially PLA<sub>2</sub> – to various carrier materials presented difficulties in the past in so far as the residual activities of the obtained preparations were below 2%. With increasing knowledge about the amino acid composition and the structure of this enzyme it was recently possible to overcome these problems. In this contribution, an immobilization method will be presented that leads to catalytically active immobilized PLA<sub>2</sub> with high operation- and storage stability. Together with a special design of the reaction mixture it is possible to enhance the activity up to more than 80 %. The obtained products can be used for the continuous production of *lyso*-phospholipids or in a flow injection analysis device for the determination of PC-concentrations. - Some new results concerning the immobilization of PLD as well as the use of lipases with phospholipase-activity for phospholipid modification will also be discussed.

1. Bornscheuer, U.T., Kazlauskas, R.J. (1999), *Hydrolases in Organic Synthesis – Regio- and Stereoselective Biotransformations*, Weinheim: Wiley-VCH.
2. Servi, S. (1999), Phospholipases as synthetic catalysts, in: *Topics in current chemistry*, Vol. 200 (Fessner, W.-D.;Ed.), pp. 127-158. Berlin: Springer Verlag.

**Tuesday, October 10, 2000, 14:30 (Biochemistry and Bioengineering)**

**Enzymatic-catalysed Enrichment of Phospholipids in Buttermilk:  
Optimisation of Reaction Conditions.**

Dagmar BENNEWITZ\*, Michel LINDER\*\*, Jacques FANNI\*\*, Michel PARMENTIER\*\*

\*Hochschule Anhalt, Köthen, Germany

**\*\*Laboratoire de Physicochimie et Génie Alimentaires**

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Buttermilk is a by-product of the dairy industry which is not well valorised today. Up to here, it was only used as beverage in few countries or used as a full additive to a number of pastry formulations. The major interesting functional property of buttermilk lies in its relatively high content in phospholipids (about 1.5 g/l). This lipid class is known to be very difficult to separate by industrial process.

The aim of this work was to concentrate the fat content in the buttermilk fraction by a membrane process after enzymatic treatments. This step might allow a better separation of the lipids from hydrolysate. To optimise the reaction conditions, a Doehlert matrix was used to study the effect of temperature (5 levels ranging from 50 to 62°C), dry matter (3 levels ranging from 60 to 100 g/l starting from spray-dried buttermilk) and enzyme/protein ratio (7 levels ranging from E/S: 0.05 to 0.35%). In this process, the hydrolysis degree (DH%) Y1, and fat concentration yield Y2, were evaluated. For this purpose, a second-order polynomial quadratic model permitted to optimise the process by using response surfaces. Reaction kinetics were monitored by measuring the DH% using the pH-stat method, osmolality and cryoscopy methods. After two hours of reaction time at 45°C and pH 8.0, DH values ranging from 15.8% up to 22.8% might be achieved with Alcalase 2.4L. After enzyme inactivation, the fat was concentrated by filtration using a regenerated cellulose membrane. Results showed an enrichment of fat (6.5 folds) under optimal conditions (0.2% E/S, 100 g/l dry matter, 54°C).

The physical and chemical properties studied such as surface tension and emulsion stability, permitted to expect several industrial applications as ingredient in the formulation area.

**Tuesday, October 10, 2000, 15:00 (Biochemistry and Bioengineering)**

### **Enzymatic-catalysed Enrichment of $\omega$ -3 Polyunsaturated Fatty Acids of Salmon Oil: Optimisation of Reaction Conditions.**

Michel LINDER, Jacques FANNI, Michel PARMENTIER

Laboratoire de Physicochimie et Génie Alimentaires

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Extraction and concentration of polyunsaturated fatty acid from salmon oil (*Salmo salar*) by enzymatic hydrolysis were studied. Enzymatic aqueous extraction of oil with Neutrase 0.5L and Alcalase 2.4L were applied to the salmon flesh (18 kg) and 45 kg of by-products (head) in batch reactor. Reaction kinetics were monitored under nitrogen by measuring the degree of hydrolysis (DH%) using the pH-stat method, in order to preserve the functional and nutritional values of hydrolysates. After two hours of reaction time at 45°C and pH 7.0, DH values of 10.4% and 17.6% could be achieved respectively with Neutrase and Alcalase. Lipids were separated by centrifugation and filtered through a column filled with anhydrous sodium sulphate with a yield of 13.8% (w/w) for the flesh and 14.3% (w/w) for the by-product, compared to 15.2% (w/w) obtained using the classical method with solvent. Lipase hydrolysis by Novozym SP 398, a specific *sn*-1, *sn*-3 enzyme, and membrane filtration, were evaluated as a mean of selectively concentrating PUFA's fractions. A Doehlert matrix was used to study the effect of reaction time U1 (5 levels ranging from 0.5 to 24 h), the flow rate U2 (90 to 750 mL/min) and the enzyme/protein ratio U3 (E/S: 0.2 to 0.6%). Quadratic models were used to generate response surfaces of the liberation of fatty acids during the reaction. Composition of the major fatty acids after esterification using the boron trifluoride (BF<sub>3</sub>)/methanol and KOH/methanol methods were determined as well as lipid classes. After lipolysis under nitrogen, the filtration step led to an enrichment from 12% in the crude oil to 31% in the permeate for docosahexaenoic acid (DHA), and from 6.0% to 10.4% in the retentate for eicosapentaenoic acid (EPA). In the same way, differential scanning calorimetry showed different melting peaks from -42.36°C to +6.85°C for the crude oil, and an exothermic event with a melting peak at +34.6°C for the glyceridic fraction in the retentate.

**Tuesday, October 10, 2000, 13:30 (Analytical Methods and Management)**

### **Analysis of Complex Sphingolipids by HPLC-ESI-MS/MS**

Hans-Ulrich Humpf

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Sphingolipids are the most structurally diverse, as well as complex, class of lipids, which are found in all cellular membranes, lipoproteins and other lipid-rich sources.

Sphingolipids are highly bioactive compounds and they are used by cells to regulate cell growth, differentiation, apoptosis and other cellular functions.

The methods which are available for the analysis of complex sphingolipids comprise hydrolysis and characterisation of the sphingoid bases, the fatty acid moieties as well as the headgroups (e.g. sugars) by high-performance liquid chromatography (HPLC) or gas chromatography (GC) after derivatisation. Since these procedures are time consuming and structural information is lost due to hydrolysis, we developed an HPLC – electrospray-ionisation tandem mass spectrometry (ESI-MS/MS) method for the analysis of intact sphingolipids. Under electrospray conditions sphingolipids could effectively be transformed into protonated molecular ions  $[M+H]^+$  and almost no fragmentation was observed. In the MS/MS mode, low energy collision-induced dissociation of the protonated molecular ions  $[M+H]^+$  with argon as collision gas produced characteristic product ion spectra which can easily be used to identify sphingolipids. The combination of HPLC and electrospray-ionisation tandem mass spectrometry provides highly structure specific data for the identification and characterisation of sphingolipids in the low picomole range.

In this paper several examples for the characterisation of different species of ceramides and cerebroside are presented. Furthermore the data for the identification of unknown compounds from natural sources are shown.

**Tuesday, October 10, 2000, 14:00 (Analytical Methods and Management)**

**Is it possible to use an electronic nose (EN) for the detection of sensorial defects in virgin olive oil?**

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We have been working with a FOX apparatus (Alpha MOS – France) since the beginning of 1998. The aim of our study was to determine if EN can help a sensory panel for a first screening of virgin olive oils in order to detect samples with sensorial defects.

Sensory assessment

European Community introduced in 1992 sensory assessment based on the IOOC method for the quality control of olive oils. The profile sheet used is divided in two parts : descriptors of quality (olive fruity, bitter, pungent) and descriptors of defects (musty, fusty, rancid, sour). An overall mark is given to the sample (from 9 to 1) according to the fruitiness intensity and the maximum defect intensity. In the international trade standard applying for olive oils, products are classified in four groups depending on their sensorial characteristics and free acidity. ITERG's panel does participate to interlaboratory studies organised by IOOC.

In order to test EN, nearly 80 samples have been collected and evaluated by ITERG's panel according to CEE2568/91 method, and by EN.

**Analytical conditions**

Static headspace is generated during 15 minutes at 50 °C with an autosampler HS 500, in 10 ml vials ; 2,5 ml of the gas phase is injected in the EN cells (2 x 6 metal oxide sensors - MOS). Synthetic air (250 ml/min) and conditioning unit (ACU500) are used. Data expressed as : maximum percentage change in resistance ( $\Delta R/R_0$ ).

**Results with EN**

Results have been analysed using a multivariate technique, principal component analysis (PCA). It has been impossible to get a good discrimination between samples of the different quality classes : "extra", "fine", "semifine" and "lampante". Even working with the two extreme classes ("extra" and "lampante"), no territory of the "lampante" oils could be defined.

Moreover, when diluting "lampante" samples in two different "extra" virgin olive oils, sensors are more sensitive to the differences between the two "extra" oils than those between sensorial defects.

HS/ GC results

To understand EN results, partition coefficients in olive oil/air at 50 °C have been evaluated. At equilibrium, volatiles such as t-2-hexenal, hexanal, 3-methyl-butanol or 1-octene-3-ol are mainly found in the liquid phase, while alcohols (methanol, ethanol), pentane and ethyl acetate can be found in the gas phase. These results explain why chromatograms of "extra" and "lampante" oils are scarcely different.

Concentrations of volatiles of interest (t-2-hexenal, hexanal, 3-methyl-butanol or 1-octene-3-ol) are too small in static headspace to be detected by either GC or EN.

**In conclusion, EN coupled with static headspace is not able to detect sensorial defects of virgin olive oil.**

Tuesday, October 10, 2000, 14:30 (Analytical Methods and Management)

## Characterization and Determination of unusual conjugated linoleic acid (CLA) isomers

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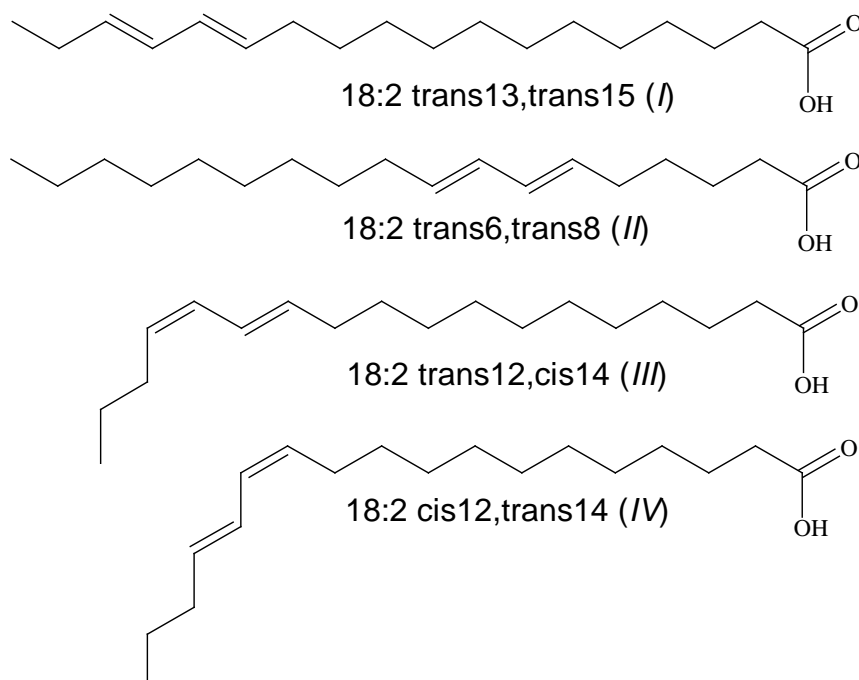
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During alkali isomerization of linoleic acid (18:2 cis9cis12, LA) a multiplicity of geometrical and positional LA isomers are formed depending on the experimental conditions (e.g. solvent, reaction temperature). The predominant isomers in these mixtures are 8,10; 9,11; 10,12 and 11,13-18:2 isomers with cis,cis; cis/trans and trans,trans double bond configuration. All these usual isomers occur also in natural products (e.g. dairy products and beef) as biohydrogenation intermediates of LA.

Recently we identified a novel minor CLA isomer – 18:2 trans7,cis9 – in cheese samples. In the present study we characterize for the first time four novel CLA isomers, namely 18:2 trans13,trans15 (*I*), 18:2 trans6,trans8 (*II*), 18:2 trans12,cis14 (*III*), and 18:2 cis12,trans14 (*IV*), by means of gas chromatographic, silver-ion high performance liquid chromatographic, and mass spectrometric techniques using fatty acid methyl ester and dimethylloxazoline derivatives. The isomers, which were formerly tentatively identified in cheese samples, were found in an alkali isomerized LA mixture. Since individual isomers have different physiological properties (e.g. 18:2 cis9,trans11 and 18:2 trans10,cis12 play distinguished roles in cancer inhibition and lipid metabolism, respectively) these results may contribute to the approach of investigating the physiological effects of individual CLA isomers in complex mixtures.





**Tuesday, October 10, 2000, 15:00 (Analytical Methods and Management)**

**Physical Refining of Coconut Oil : Effect of Crude Oil Quality and  
Deodorisation Conditions on Neutral Oil Loss**

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Neutral oil loss (distillative and mechanical carry-over) during physical refining of coconut oil was quantified. Neutral oil loss seems to depend on both crude oil quality and the process conditions during deodorisation. The distillation of volatile glyceridic components (mon- and diglycerides,...), originally present in the crude oil, was confirmed as the major cause for the neutral oil loss. The amount of these volatile components in crude coconut oil cannot be derived as such from the initial free fatty acid content.

A 'deodorisability' test on lab-scale under standardized conditions (temperature = 230°C ; pressure = 3 mbar, time = 60 min. and sparge steam = 1%) is described to evaluate the crude oil quality and to obtain a more accurate prediction of the expected neutral oil loss and free fatty acid content in the distillate.