Abstracts of papers

Unless indicated by *, first name given is speaker.

1

WHAT'S NEW IN FATS & OILS PROCESSING. J.V. Landis, Anderson Clayton, P.O. Box 2538, Houston, TX 77001.

We are still trying to recover pure triglycerides and throw the balance away. We present some provocative questions on why we still process fats using essentially the same techniques our fathers used. In view of our environmental and energy problems we need some innovative R&D.

2

PHYSICAL REFINING OF AFRICAN MAIZE OIL. A Forster and H.J. Nel, Simon-Rosedowns Ltd., Cannon Street, Hull, England.

The use of 'physical' or 'non-caustic' refining of certain edible oils and fats is now well established. Such systems for processing the relatively high F.F.A. palm oil are particularly well documented and it is common for similar systems to be applied to the 'Lauric Oils' of coconut and palm kernel. Such systems are not, however, in general use for the common liquid oils. Their generally low F.F.A. and the more rigorous pre-treatment systems necessary for good quality product tend to cause hesitation by the refiner in opting for the non-caustic method of refining these oils. An exception to this latter statement is African Maize Oil which can vary usually from 5 to 10% and frequently 15% F.F.A. Refining factors of more than twice the F.F.A. can be incurred during caustic refining of this oil. With physical refining factors of 1.1 to 1.2 times the F.F.A. are possible. The development of a physical refining route for this oil has therefore been well justified on economical grounds alone. The paper briefly discusses the background to production of high F.F.A. maize oil and the development of a physical refining process to improve previous economics and provide a good quality oil. Detailed discussion is afforded to the description of a fully operational 100 T.P.D. plant and a survey of the process, process equipment, operating parameters and product quality assessment. The application of this process and plant to sunflower oil is briefly discussed.

3

TECHNICAL CONSIDERATIONS ON THE FAT SPLITTING, DISTILLATION, FRACTIONATION, AND HYDROGENATION PROCEDURES. E. Bernardini and M. Bernardini, Costruzioni Meccaniche Bernardini, 00040 Pomezia (Roma), Italy.

This paper covers the various stages of splitting, distillation, fractionation and hydrogenation of fats and oils for the production of fatty acids and glycerin. From the combination of the above-mentioned procedures and from the monitoring of the process variables it is possible to obtain a wide range of products, which find application in many industrial sectors (detergents, cosmetics, pharmaceutical products and the like) and which in some cases are a valid substitute of petroleum derivatives. A series of process flow-sheets and diagrams illustrate the operation of the various stages which constitute the subject plants.

4

THE IMPACT OF THE PREPARATION AND EXTRACTION CONDITIONS OF SOYBEANS ON THE OIL QUALITY. T.L. Ong, Central Institute for Nutrition and Food Research, Post Box 360, 3700 Aj Zeist, Holland.

Soybeans contain enzymes, particularly lipoxygenases and phospholipases that lower the oil quality. By inactivation of these enzymes prior to flaking and subsequent extraction, soybean oils of high quality can be obtained; these oils have low oxidation values, very low nonhydratable phospholipids (10-20 ppm P) and iron (1.0–1.5 ppm Fe) content, and are very well suited for steam refining. Concurrently, with the inactivation of the enzymes by means of heat treatment, the PDI of the meal is lowered, making the material unsuitable for preparing protein isolates.

5

TIRTIAUX FRACTIONATION: THE FLEXIBLE WAY TO NEW FATS. Alain Tirtiaux, S.A. Fractionnement Tirtiaux, 601, Chausée de Charleroi, B-6220 Fleurus Belgium.

Natural fractionation provides a simple and economical solution to the problem of splitting most edible fats and oils into several products. The Tirtiaux process, with its accurate crystallization control, and long industrial experience, allows a choice of crystallization conditions and separation temperatures to ensure the obtainment of products of specific quality at low cost. This is the result of a technique developed by Tirtiaux which consists mainly in the formation of suitable crystal seeds and the control of their growth by regulating the heat transferred from fat to the coolant. The choice of the separation temperature and the ability to refractionate any one of the end products give a wide range of possible qualities. The separation is done on the Tirtiaux florentine continuous vacuum filter equipped with a stainless steel perforated belt as filtration support. A recycling device for any crystals sucked through the belt at the edge of the horizontal vacuum surface ensures a filtration on a preformed cake. The coarse mesh of the belt together with the large size of the crystals obtained allow an easy filtration with low vacuum even if the viscosity of the oil is high. The filter is therefore able to operate on delicate crystals as those obtained when fractionating hydrogenated soybean and fishoil or when refractionating palm olein at low temperatures.

6

DETERGENT FRACTIONATION OF FATS AND OILS. Alan McCabe and Hans Nilsson, Alfa-Laval AB, Box 500, S-147 00 Tumba, Sweden.

While the detergent fractionation process has been in use in the fats and oils industry for many years, there is a tendency to assume that its only application is for the fractionation of palm oil. Although it is true that palm oil fractionation has been one of the main uses for the process, it should be known that many other fractionation/winterization processes can be carried out using the detergent fractionation principle. The paper will present the basic principle of the process and also its application in full scale operation. Data from operating plants will be given together with data from either lab scale or pilot plant for a series of applications for the process.

7

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF THERMALLY OXIDIZED OLIVE OIL TRIGLYCERIDES. E.G. Perkins and Ali El-Hamdy,* 205 Burnsides Research Laboratory, University of Illinois, Urbana, IL 61801.

Separation of triglycerides has been achieved using high performance reversed-phase chromatography (HPRC) which has recently emerged as a powerful technique in lipid analysis. In this work a combination of high performance gel permeation (HPPC) and HPRC were used to detect the changes that occur as a result of heating olive oil. Olive oil was used for deep fat frying at 215 C for 10 days, 8 hours a day. French fries were prepared every 30 minutes. The thermally oxidized olive oil samples were separated according to molecular weight using HPPC on a 2 x 500 Å and 2 x 100 Å μ styragel columns using tetrahydrofuran as the mobile phase. Fractions of less than 1000 daltons molecular weight were collected and separated on HPRC using 5 μm Supelcosil LC-18 columns. The components were collected and analyzed by mass spectrometry. The higher molecular weight triglyceride fractions were esterified and separated on HPRC and analyzed by mass spectrometry. Another sample of the thermally oxidized olive oil was esterified, urea-addicted to concentrate the oxidation products, separated on HPRC and analyzed by mass spectrometry for the presence of cyclic monomers.

8

THERMALLY INDUCED CYCLICS AND AROMATICS IN FATS AND FAT MODEL SYSTEMS. Pio Angelini, Analytical Chemistry Branch, Food Science Laboratory, U.S. Army Naradcom, Natick, MA 01760.

Triglycerides containing either saturated or unsaturated fatty acids and fats from both animal and plant origin were heated to different temperatures for different lengths of time under high vacuum or in an inert atmosphere. The resulting thermolysis products were separated by low temperature-high vacuum distillation (LT-HU), gas chromatography (GC) and high pressure liquid chromatography (HPLC), and characterized by mass spectrometry (MS). The effects of temperature, and time of heating on the model systems and natural fats are reported in terms of visual observations and chemical analyses of the thermolytic products. Both the identity and relative amounts of the thermolytic products, with special emphasis on cyclic and aromatic compounds, are discussed with respect to original composition and heating parameters.

9

THE INFLUENCE OF FEEDING ABUSED FATS ON THE INTESTINAL ABSORPTION OF ^{14}C-THIAMIN IN THE RAT. E.G. Perkins and Jane Lai, Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, IL 61801.

Refined, bleached and deodorized soybean oil was abused by frying potatoes each half hour for twelve hours each day for a total of five days. Eighteen treatments of five rats, each involving 10, 20,

and 30% protein levels, and three levels of thiamin, were fed heated and fresh oil for a total of six weeks. Weight gain, feed efficiency and protein efficiency ratios were determined. The rats were killed and the liver body weights ratio determined, as well as the lipid content of the liver. Significant differences were found in the liver size and growth of the rats fed the low protein levels. At this low protein level the feeding of thiamin at higher levels did not alleviate the effect. Everted intestinal sacs were prepared from freshly killed animals and the transport efficiency of thiamin determined. No significant influence of feeding the heated oil on the absorption of thiamin was found.

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ANALYSIS OF THERMALLY ABUSED SOYBEAN OIL FOR CYCLIC MONOMERS. *Joyce* B. Meltzer, Edward G. Perkins, E.N. Frankel and T.R. Bessler, Department of Food Science, University of Illinois, Urbana, Illinois 61801.

Cyclic monomers derived from the intramolecular condensation of the C_{18} polyunsaturated fatty acids have elicited toxic responses when fed to laboratory animals at low dietary levels. The present study was undertaken to quantitate the cyclic monomers formed by the thermal oxidation induced during the deep-fat frying process in order to assess the potential toxicity of oils abused in this manner. Two separate experiments were designed to study the effect of unsaturation and type of heating on cyclic monomer formation. Three hundred grams of potato slices were fried at 30 min intervals in 3 liters of soybean oil and hydrogenated soybean oil (IV 107) maintained at 195 C in a household deep-fat fryer. Appropriate amounts of make-up oil were added at the end of each 8-hr period. A total of 73 pounds of potatoes were fried over a period of 104 hr under both continuous and intermittent heating conditions. IV decreases in the range of 10-15 units and 5- to 10-fold increase in FFA indicated that the heated oils had sustained significant chemical and physical alterations. Selected samples were catalytically hydrogenated and analyzed for cyclic monomers by GLC. At chromatographic conditions sensitive enough to detect a cyclic monomer standard at concentrations lower than 2% by weight, no cyclic monomers were detected in any of the heated oil samples. However, when the samples were concentrated by removing a major portion of the saturated fatty acids by low-temperature crystallization, levels below 0.3% of cyclic monomers were detected in heated soybean oil. Although no feeding studies were made with these oils, we believe, on the basis of previous studies (Iwaoka and Perkins, Lipids 11:349, 1976) with laboratory animals, that a level of cyclic acid greater than 1% in the oils would be necessary to produce toxic responses.

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EVALUATION OF GROUNDNUT AND COTTONSEED OILS FOR DEEP-FAT FRYING. Lourens M. Du Plessis, Pieter van Twisk, Pieter J. van Niekerk and Martin Steyn, National Food Research Institute, P.O. Box 395, Pretoria 0001, South Africa.

A comparative study of cottonseed oil and groundnut oils for frying of potato chips was undertaken. Industrial scale frying was conducted for 5 days with cottonseed and 5 days with groundnut oil, and frying oils and chips were sampled twice a day. Frying oils and oils extracted from stored chips were analyzed for ultraviolet absorption (A_{232} and A_{268}), peroxide and acid values. Tocopherol and tertiary butylhydroquinone levels were determined by high performance liquid chromatography. Chips stored at room temperature for 12 weeks were organoleptically evaluated. During the first 10 h frying the A_{232}, free acid and peroxide values of cottonseed oil increased rapidly, exceeding that of groundnut oil, which increased moderately. For both oils constant values were attained during the next 70 h period, followed by moderate increases during the last 23 h. Groundnut frying oil lost 55% of its tocopherols and 54% of its tertiary butylhydroquinone during frying (103 h), while cottonseed frying oil retained these compounds at the original levels. Tocopherols were also better retained in chips fried in cottonseed oil than in groundnut oil. The fatty acid patterns of frying oils and oils extracted from chips did not show significant changes due to frying and storage, respectively. These results, therefore, suggest that cottonseed oil is sufficiently stable to be used as a substitute for groundnut oil in deep frying.

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QUICK ASSESSMENT OF FRYING OIL QUALITY BY COLUMN CHROMATOGRAPHY. A.K. Sen Gupta and G. Guhr, Unilever Forschungsgesellschaft mbH, Behringstr. 154, D-2000 Hamburg 50 Germany.

A column chromatrgraphic method, which was earlier developed for the analysis of polar lipid mixtures and of oils, has been adapted for the quality assessment of used frying fats. The method is based on the separation of a frying fat into a fraction containing unchanged triglycerides by using a silica gel column. The polar products which are produced from the triglycerides due to thermal and oxidative changes occurring during the frying can thus be determined easily by difference. This method can easily be practiced in any laboratory by using simple equipment. Two other methods, viz. Gel Permeation Chromatography and High Pressure Liquid Chromatography, together with the new column chromatographic method were compared with the classical standard method of determination of the petroleum ether insoluble oxidized fatty acids of used frying fats. The results will be presented. In addition to the advantage of using simple equipment the time requirement for the new assessment method is quite small. It yields well reproducible results in routine assessment of used frying fats. It has been therefore suggested to be used as a standard method by the German Society for Fat Research. At the moment it is being considered by the IUPAC to be recommended as a standard method for the quick assessment of used frying fats.

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FOOD SAFETY FROM A COMPANY LAWYER'S VIEWPOINT. Altred E. Johanson, Foremost-McKesson Inc., 155 E. 44th St., New York, NY 10017.

Abstract unavailable at press time.

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RESIDUE CONTROLS IN IMPORTED MEAT AND POULTRY. H.M. Steinmetz, U.S. Department of Agirculture, Food Safety and Quality Service, MPI-FPS, Room 4346-So. Bldg. 14th & Independence Ave. S.W., Washington, DC 20250.

The Federal Meat Inspection Act and the Poultry Products Inspection Act provide for the protection of the health and welfare of consumers by assuring that meat and poultry products distributed to them are wholesome, not adulterated and properly marked, labeled and packaged. The responsibility for carrying out these Acts is delegated to the Department of Agriculture. The regulation by the Department applies to articles in interstate or foreign commerce and provides for cooperation with the States with respect to articles in intra-state commerce. The Acts' definition of "adulterated" includes bearing or containing a pesticide chemical, food additive, and color additive unsafe within the meaning of the Food Drug and Cosmetic Act. Meat and poultry may be imported into the United States only from countries specifically approved on the basis of a "national" inspection system providing inspection standards and requirements, equivalent to U.S. requirements, in plants preparing product for export to the United States. Repeated on-site compliance reviews of all foreign plants eligible to export to the United States are conducted by Review Officers of the Department's Meat and Poultry Inspection Program. Each shipment of foreign meat or poultry is subjected to a port of entry inspection before it is permitted entry into U.S. Commerce. Under the "equal to" requirements of the laws, each eligible foreign country is required to carry out a residue control program in plants exporting to the United States. Residue samples are collected by USDA inspectors from shipments of products proposed for entry from all eligible countries. Violative results are the basis for rejection and reexportation of shipments. Continuing problematical results can lead to the removal of a plant's eligibility to export and indications of a country-wide problem can result in removing the eligibility of all plants in an otherwise eligible country.

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AFLATOXIN CONTROL—PAST, PRESENT, FUTURE. Leonard Stoloff, FDA, HFF-454, 200 C St., SW, Washington, DC 20204.

Domestic commodities most susceptible to aflatoxin contamination are peanuts, corn, cottonseed, and tree nuts (almonds, pecans, walnuts); the most susceptible imported commodities are Brazil nuts and pistachio nuts. The development, effectiveness, and shortcomings of the strategies used to limit consumer exposure to aflatoxin from these commodities are reviewed.

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TOXICITY TESTING GUIDELINES: ROLE IN RETROSPECTIVE SAFETY REVIEWS. Victor H. Morgenroth III, Food and Drug Administration, 200 C Street, S.W. (HFF-156), Washington, D.C. 20204.

The Interagency Regulatory Liason Group (IRLG) was established for the purpose of improving public health through sharing of information, avoiding duplication of effort, and developing consistent regulatory policy. The IRLG agencies established the Testing Standards and Guidelines Work Group for the purpose of developing guidelines which would satisfy the toxicity testing needs of its member agencies and resolve current differences in testing requirements. The work group has reviewed the tests, guidelines, and procedures, in use or under development, sought outside advice and attempted to coordinate its work with others in the process of guideline development. The work group's first draft guidelines on acute toxicity tests were made available to the public in August of 1979. A number of retrospective toxicological reviews of chemical safety are being performed by IRLG member agencies. The cyclic review of direct food additives in the Bureau of Foods at the Food

and Drug Administration is an example of one of these retrospective safety reviews. The assessment of the adequacy of toxicity data is an important feature of these retrospective safety reviews. This assessment involves the application of toxicity testing standards or guidelines such as the IRLG Guidelines to data generated during the past 25 years. The difficulty in the application of "state of the the art" guidelines to old data is apparent. This paper discusses the development of the "Core Standard" approach for determination of the adequacy of data for direct food additive cyclic review.

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FDA REGULATIONS TO ASSURE FOOD SAFETY. Charles J. Kokoski, Food and Drug Administration, 200 C Street, S.W. (HFF –156), Washington, D.C. 20204.

The consumer wants assurances that his food supply is wholesome and safe. We have seen technological advances in food production and distribution to meet the demands of an ever-increasing population and changing patterns of living. We have seen a greater dependence upon commercial processing of food. The technological advances over the last several decades have brought into use many new chemicals and substances which are useful in meeting the consumer demands that there be an abundant, wholesome and tasteful variety of foods. Along with this, the consumer demands that his food be safe. He looks to the government to assure this safety. Significant advances in Federal regulatory authority over food safety came in the 1950–60 period with Congressional passage of the Pesticide Amendments in 1956, the Food Additives Amendment in 1958 and the Color Additive Amendments in 1960. The burden of demonstrating safety rests with industry, but the FDA has the responsibility of evaluating the data presented by a petitioner who requests a new food or color additive and of making a determination if a regulation shall be issued establishing the safe conditions of use. This process involves a detailed scientific evaluation of all data and information bearing on safety of the additive. Over the years we have seen an evolvement of the criteria and concepts in toxicology testing and evaluation of additives in food. Requirements for supporting safety are more extensive today. This has resulted in a need for a systematic re-evaluation of the safety data base supporting food and color additive regulations written over the past years.

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SOLIDIFICATION BEHAVIOR OF PALM OIL. M. Naudet and E. Sambuc,–Laboratoire National des Matières Grasses–ITERG Université d'Aix-Marseille–Pl. V. Hugo–F 13331 Marseille Cedex 3 (France).

Palm oil is a so-called "slow solidifying fat." Its behavior during solidification is easily studied using width band NMR in convenient conditions. This behavior for a refined and bleached palm oil is characterized essentially by an initial overmelting of low intensity and by a secondary, less important overmelting which appears when the temperature of the oil has reached 10 C. Initial temperature has no influence so long as it is not lower than the clear melting point, and is maintained, before cooling, a sufficient time. Dispersed water has no visible influence upon the beginning of the solidification, but enables the disappearance of the secondary late overmelting. Additions, like partial glycerides or lecithins, have various influences according to whether they are used in the presence of water or not.

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THE NUTRITIVE VALUE OF PALM OIL. J.J. Gottenbos and R.O. Vles, Unilever Research, Vlaardingen, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands.

The nutritive value of palm oil and its fractions has not been studied systematically up to now. But, in the course of our short- and long-term studies into the biological effects of edible oils with respect to absorption, growth, organ functions and tissue pathology in various animal species, palm oil was occasionally included. In most of these experiments, palm oil feeding lead to results which could be expected from its fatty acid composition. However, in experiments with high erucic acid rapeseed oils, adding palm oil to the diet of guinea pigs and ducklings alleviated most of the pathological effects of erucic acid. In long term studies with rabbits fed cholesterol-free diets, palm oil proved to be atherogenic as would be expected from its low P/S ratio. This atherogenic effect could be counteracted by mixing palm oil with oils rich in linoleic acid. The role of palm oil in human nutrition will be evaluated in comparison with other edible oils and fats.

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THE CO-DOMINANCE THEORY: GENETICAL INTERPRETATIONS OF ANALYSES OF MESOCARP OILS FROM *ELAEIS GUINEENSIS, ELAEIS OLEIFERA* AND THEIR HYBRIDS. Augustine Ong Soon Hock, Chuah Chong-Cheng and Sow Huan Pauh, School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia.

Determination of the fatty acid and triglyceride compositions of the F_1 (*Elaeis guineensis* x *Elaeis oleifera*), F_2 and the backcross hybrid mesocarp oils demonstrated that most fatty acid and triglyceride compositions of oils from hybrid palms are intermediate between those of their respective parentals. These data as well as the acyl group (saturated/palmitate and unsaturated/oleate) distribution of triglycerides of the F_2 mesocarp oils provide genetical proof for co-dominance in the F_2 generation which shows a characteristic segregation into the co-dominance ratio of 1:2:1 (i.e., 1 *E. guineensis*:2 F_1 hybrid:1 *E. oleifera*). Similar analyses into the backcross hybrid mesocarp oils on the whole confirmed co-dominance when the backcross ratio of 1:1 was obtained. The above results are used to develop the Co-Dominance Theory of *Elaeis* Palm Hybridization which makes successful predictions for mesocarp oils from the different hybrid palms.

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THE PALM OIL RESEARCH INSTITUTE OF MALAYSIA–A BEGINNING. K.G. Berger, 18th Floor, Angkasa Raya Building, Jalan Ampang, Kuala Lumpur, Malaysia.

The Palm Oil Research Institute of Malaysia (PORIM) is a national body, legally established in May 1979, and financed by a research cess on palm oil exports. It is managed by a statutory board comprising government and industrial representatives. PORIM is charged with the task of conducting and promoting research into production, extraction, processing, storage, transport, marketing, consumption and end uses of palm oil products. It will also provide a technical advisory service and an information service for palm oil users. The paper describes the setting up of the organisation, its temporary laboratories and some preliminary research findings.

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TECHNICAL COLLABORATION IN THE MALAYSIAN OIL PALM INDUSTRY. D.A.M. Whiting, and K.G. Berger, Malaysian Oil Palm Growers' Council (Technical Research Committee), G.P.O. Box 747, Kuala Lumpur 01-02, Malaysia.

The Malaysian Oil Palm Growers' Council (MPOGC) is an Association of oil palm growers and palm oil extractors. It is active in technical, agricultural and commercial matters. This paper describes some results of collaboration carried out by its Technical Research Committee (TRC). Analytical tests for the following parameters of crude palm oil have been studied: free fatty acids (FFA), volatile matter (VM), Impurities (I), Peroxide value (PV), and anisidine value (AV). A statistical evaluation of the results is presented, and some improvements to the test methods are proposed. Work is proceeding to improve and standardize tests for trace metal contents of crude palm oil. The Malaysian Agricultural Research and Development Institute (MARDI) has, on behalf of the MOPGC-TRC, carried out a study of the identity characteristics of Malaysian crude palm oil. A comprehensive series of samples was drawn from extraction mills and bulking installations. Results are presented. The studies on density have been extended so that a standard density/temperature chart is now available. The TRC reviewed process control test methods for the extraction process, as a result of which standard guidelines have been published. The TRC also organized three symposia on effluent treatment and disposal as part of its efforts to meet government standards for effluents. Some of the significant findings are described.

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PALM OIL: PAST–PRESENT–FUTURE. B.J. Jacobsberg, and M. Loncin, Ceria IIF-IMC, Technologie Alimentaire I, av.E.Gryson, 1070 Bruxelles–Belgium.

Today's production expansion of palm oil has been made possible thanks to a better understanding of enzymatic activity in the fruit and chemical reactions occurring in the oil during and after fruit processing. Improved handling and processing of palm fruit has led to the production of high quality palm oil. Problems in refining and processing of palm oil are discussed in relation to oil characteristics and quality in terms of analytical data. Its range of distinctive properties enables its use in a large number of edible products. Questions remain open for further research, such as in quality forecast, separation and purification of its constituents, etc.

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RECENT PROGRESS IN THE HPLC OF LIPIDS. K. Aitzetmüler, Unilever Forschungsgesellschaft mbH, D 2000 Hamburg 50, West-Germany.

Recent developments in the HPLC of lipids are highlighted and discussed. New trends and application areas are identified and commented, and the published literature is reviewed. Cautious forecasts are given in an outlook to the future as seen by the author. In separation methodology, newer developments include an increased use of reversed-phase HPLC for homologue separations, non-auqeous reversed-phase, ion pairing techniques, including "reversed pairing," and a renewed interest in silver nitrate-impregnated columns and other column-solvent systems exhibiting

special selectivites. Preparative HPLC is coming of age particularly in those areas that are of biomedical interest, as with prostaglandins, steroids and phospholipids. Class or group separations are frequently used for semipreparative purposes, e.g., to obtain fractions suitable for subsequent analysis by a different chromatographic process. On the detector side, the current lack of a universal, lipid-sensitive HPLC detector that can be used with gradient elution has led to a variety of attempts to achieve the desired results by other means, e.g., pre-fractionation plus isocratic HPLC, UV and fluorescence derivatization, short-wavelength UV, light scattering, infrared, polarographic, fluorescence enhancement, mass spectrometric detection, etc. In the field of lipids and related substances, certain "clusters" of HPLC activity can be identified, e.g., prostaglandins; lipid soluble vitamins; insect-pheromones; partial glycerides and emulsifiers; polar (glyco- and phospho-) lipids of biomedical interest; triglycerides, including edible oils and their oxidation products; fatty acid separations (as UV- or fluorescent derivatives); and oxidation products of unsaturated fatty acids.

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THE USE OF HPLC IN ANALYSIS OF A TRIGLYCERIDE MIXTURE. Bengt Herslöf, AB Karlshamns Oljefabriker, S-292 00 Karlshamn, Sweden.

The separation of triglyceride mixtures on HPLC have been investigated by several groups. Our laboratory has also been working in this area and our experience in this field are summarized in this paper. It includes comments on columns, eluents, solvents etc. The HPLC work has been combined with other separation techniques in order to obtain as much information about the individual triglycerides as possible. Fractions collected from semi-preparative HPLC have been submitted to further separation on GC and Argentation-TLC. Results from investigations carried out on some vegetable oils are presented.

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ANALYSIS OF LIPIDS VIA A NEW LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY COMPUTER SYSTEM. W.L. Erdahl, W.R. Beck, D.E. Jarvis and O.S. Privett,* The Hormel Institute, University of Minnesota, Austin, MN 55912.

Apparatus and techniques are described for the analysis of lipids by a LC-MS-COM system. The interface system for coupling the liquid chromatograph to the mass spectrometer is based on the endless chain principle in which a belt of special construction is used as the transport device. After removal of the solvent, the sample is transported on the belt into a reactor where it is evaporated if it is volatile, or converted to hydrocarbons if it is not volatile and introduced into the source of mass spectrometer in a carrier gas. Operation and performance of the system is demonstrated by the analysis of model compounds representing the common lipid classes. A ramification of the interface is its use as an injection system for direct chemical ionization mass spectrometry-computer analysis (IF-MS-COM). Application of this mode of operation as a rapid method for determination of fatty acid composition is described.

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF STEROIDS. Erich Heftmann and I.R. Hunter, Western Regional Research Center, Science and Education Administration, U.S.D.A., 800 Buchanan St., Berkeley, CA 94710.

Work in this laboratory on the application of HPLC to the following classes of steroids is reviewed: sterols, ketonic sterols, androgens, pregnane derivatives, sapogenins, alkaloids, and withanolides. Relations between steric configuration and chromatographic behavior are discussed.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF BILE ACIDS. William H. Elliott and Roger Shaw, Department of Biochemistry, St. Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104.

HPLC has been used to separate a few conjugated 5β-bile acids by several groups of investigators with various aqueous solvent systems. We have utilized a Waters Model ALC 201 instrument equipped with a U6K loop injector, a Model 401 differential refractometer or a Schoeffel Model 770 UV flow monitor with a μBondapak/C_{18} column (30 cm x 4 mm I.D.) to study the separation of twenty glycine or taurine conjugated of 5β- and 5α-bile acids with several solvent systems. Similarly a Waters Radial Compression Separation System has been investigated with a solvent system of 2-propanol/10 mM potassium phosphate (pH 7.0) (130:340) at a flow rate of 1 ml/min. Separations of conjugated dihydroxy bile acids of the 5β-series were attained, but separations of comparable 5β- and 5α-isomers were more difficult. From data obtained on the μBondapak/C_{18} column with these twenty conjugates the average contribution of the glycine and taurine moieties could be calculated. Similar studies with free bile acids have shown that the contributions of substituents at positions 3,6,7 and 12 and unsaturation at positions 4,6,9 and 11 can be utilized to predict mobilities of polysubstituted bile acids with a particular solvent system. These results show that a comprehensive study can provide information relative to the contribution of individual functional groups toward the prediction of mobility of polysubstituted bile acids in HPLC. (Supported by NIH Grants CA-16375 through the National Large Bowel Cancer Project and HL–07878).

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PHASE BEHAVIOR OF ALCOHOL ETHOXYLATE-OIL-WATER SYSTEMS AND ITS RELATIONSHIP TO DETERGENCY. Robert C. Pierce and James R. Trowbridge, Colgate-Palmolive Company, 909 River Road, Piscataway, NJ 08854.

The mechanism of action of alcohol ethoxylates in oily soil removal has been investigated. In a mechanically agitated wash system, rapid surfactant penetration into the oil phase and the establishment of a bi-directional surfactant flux has been shown to lower oil-water interfacial tensions to less than 1 dyne/cm. These low oil-water interfacial tensions appear to be the predominant factor correlating with oily soil removal from synthetics in the tergotometer. Oil-surfactant-water phase behavior studies indicate that surfactants giving optimum oily soil detergency have their cloud points below the wash temperature by between 20–40 C. These optimum performers are readily soluble in the oil phase and have maintained partial water solubility. Physical-chemical measurements and detergency studies employing homogeneous linear primary alcohol ethoxylates, C_{12} (1-8) EO, identify the C_{12}4EO and C_{12}5EO material as the most active in interfacial tension reduction and oily soil removal in the tergotometer.

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THERMAL REARRANGEMENT OF SULFATED TALLOW ALKANOLAMIDES, R.G. Bistline, Jr. and W.M. Linfield, Eastern Regional Research Center, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, Pennsylvania 19118.

A study of reaction variables in the sulfation of tallow alkanolamides revealed that a molecular rearrangement to aminoester occurs as a result of prolonged heating in the acidic state. Heating sulfated isopropanolamide 4 hr at 60 C resulted in 60% loss of active ingredient. The sulfated diglycolamide heated at 60 C for 12 hr suffered a loss of 22% active ingredient. A 50:50 mixture of these sulfated alkanolamides heated 12 hr at 40 C incurred no loss of active ingredient content, whereas at 60 C a 40% loss of active ingredient was observed. Thus, careful temperature control and rapid neutralization after sulfation are required to obtain sulfated alkanolamides with a high percent active ingredient.

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THE EFFECTS OF WATER HARDNESS ON SURFACE TENSION AND CONDUCTANCE PROPERTIES OF SOAP SOLUTIONS. Joan W. Koppenbrink, Armour Research Center, 15101 N. Scottsdale Rd. Scottsdale, AZ 85260.

Surface tension readings and conductivity measurements were obtained from soap solutions diluted in water with varying degrees of hardness due to calcium and magnesium. Surface tension values have indicated that the interactions which occur between the metal ions and the soaps are very complicated and involve more than a simple stoichiometric combination of ions to form lime soaps. There is evidence that each divalent cation interacts with three to four anions before it is effectively neutralized. Conductivity values on the same solutions have suggested the possibility that charged lime soap intermediates exist at critical molar ratios of soap to hardness of 1:2.

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CORRELATION OF INTERFACIAL TENSION AND DISHWASHING. T.P. Matson and G.D. Smith, Conoco, Inc., Drawer 1276, Ponca City, Okla. 74601.

Measurements of interfacial tension by spinning drop tensiometry between dishwashing soils and surfactant solutions have shown good agreement with dishwashing tests. The optimum dishwashing ratios for linear alkylate sulfonate/alcohol ether sulfate, alcohol ether sulfate/amine oxide, and alcohol ether sulfate amide blends are exactly the interfacial tension minima for these systems against the test soil. Further, the dishwashing response to hardness is also predicted quite sensitively by the interfacial tensions. The interfacial tension data appear to detect critical points in the phase equilibria of the systems which also produced pronounced foaming properties which can be detected by dishwashing. However, the minima in interfacial tensions can be much more accurately determined than foaming optima. The results must be evaluated in the light electrostatic effects since the lack of coulombic repulsions prevent nonionic surfactants from foaming very well regardless of the attendant adsorption characteristics. Insufficient data exist to determine whether this technique is useful for head to head product comparisons. It has, however, been quite accurate in predicting the foaming optima of surfactant blends.

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EFFECT OF QUATERNARY AMMONIUM SUBSTITUTION OF HYDROXYETHYLCELLULOSE ON BINDING OF DODECYLSULFATE. Kazuo Ohbu, Osamu Hiraishi, Ichiro Kashiwa and Isamu Kadoya, Lion Fat & Oil Co. Ltd., 13-12 7-Chome, Hirai, Edogawa-Ku, Tokyo, Japan.

Binding of SDS to quarternary ammonium substituted hydroxyethylcellulose (cationic cellulose) of varying degree of substitution was studied by the following measurements: (a) binding isotherms of SDS to cationic cellulose as obtained by dialysis method; (b) partial molar volume of each cationic cellulose from density measurement; (c) spin-lattice relaxation time of SDS protons in the course of binding. Binding isotherms showed the similar feature to that observed for globular protein/SDS system. The isotherm may be represented by the two distinct regions. The initial part of the isotherm follows Langmuir type in which the binding ratios of DS to ammonium residue are found to be almost one regardless of the cationic substitution degree. The result indicates that the electrostatic interaction is dominant in this concentration region. The isotherm shows further rise with the increase of dodecylsulfate concentration beyond Langmuir range. The multi-molecular binding of dodecylsulfate as observed at higher concentration suggests that the interaction being of hydrophobic nature. Partial molar volume of cationic increased in proportion to the degree of cationic substitution. Number of quarternary ammonium residues in a unit volume of solution considerably affect the dodecylsulfate binding. Spin-lattice relaxation time T_1, of SDS protons in the complex state was significantly shorter than that of SDS in micellar state.

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THE APPLICATIONS OF LECITHIN IN THE FOOD INDUSTRY. William E. Prosise, Central Soya Co., 1300 Ft. Wayne Bank Building, Ft. Wayne, Indiana 46802.

As background material, lecithin chemistry is presented with respect to governmental regulations and commercial product types. A brief discussion of manufacturing methods for lecithin products follows. Examples show how commercial lecithin types meet the technical requirements of specific applications. Additionally, general applications and lecithin's functions in each are explained. In conclusion, the future of lecithin products and their applications are explored.

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USES OF LECITHIN IN CONFECTIONERY PRODUCTS AND FOOD DRINKS. Bernard W. Minifie, Knechtel Laboratories, 26, Grange Rd., Saltford Bristol BS18 3AG, England.

Introduction. A short resumé of origin, chemistry, types of natural lecithins, synthetic phospholipids and modified lecithins. (Other lecturers will probably give more detailed information on pure chemistry). *Lecture Subjects.* Short summary of the use of lecithin in chocolate, sugar confectionery and "Instant" chocolate drinks. Emulsifying and wetting properties. Flavor effects. *Use of Lecithin in Chocolate.* Viscosity reduction, general remarks on the structure of chocolate, non-Newtonian properties, fat saving by the use of lecithin. Comparison of the effect of lecithin in chocolate, cocoa/fat mixtures, sugar/fat mixtures. Methods of incorporating lecithin, effect of excess use of lecithin. Flavour changes in milk chocolate arising from use of natural lecithins. *Synthetic Phospholipids.* "YN"–prepared by glycerolysis and phosphorylation of rapeseed oil. Other synthetic phospholipids and synergistic emulsifiers. *Plastic Viscosity, Yield Value.* Viscosity changes in chocolate due to mechanical processes. *Use of Lecithin in Powdered Drinks.* Lecithin treatment of composite powders, "instantizing." Methods of adding lecithin to powders, pre-treatment of powders before instantizing. *Summary.* Some problems to be solved with the use of lecithin in confectionery. Flavour reversion, dispersion.

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LECITHIN: THE THINGS IT DOES, CAN (OR CANNOT) DO, AND CAN BE MADE TO DO IN BAKING. Y. Pomeranz, U.S. Grain Marketing Research Laboratory, 1515 College Avenue, Manhattan, Kansas, 66502.

Lecithin, a ubiquitous ingredient of many raw materials and baked goods and an illdefined by-product of soybean oil production, has been implicated in many ways as an improver in baking. The review summarizes studies on the *functions* and *effects* of lecithin in processing, technological quality, shelf life, and consumer acceptance of baked *products*. The *functions* include physical and biochemical interactions with wheat flour components that improve dough processing stability; reduce shortening requirements, enhance its effects, and increase versatility of shortening formulations. The *effects* result in products with better volume, crumb grain, and freshness retention. The *products* include bread, toasted bread, zwieback, cakes, cookies, crackers, and waffles. Both regular and protein- or fiber-enriched products were studied; advantages in the production of non-wheat baked proucts were reported. Claims about antioxidants and nutritional value of using lecithin in baked products were recorded.

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THE USE OF LECITHIN IN RECONSTITUTING WHOLE MILK POWDER. A.J. Baldwin and W.B. Sanderson, New Zealand Dairy Research Institute, Private Bag, Palmerston North, New Zealand.

In many markets instant whole milk powder (WMP) is gaining acceptance as a convenience food. The process for the production of instant WMP consists of the formation of agglomerates, followed by the coating of these particles with a surfactant to overcome the hydrophobic nature of the fat in the powder. In a survey of surfactants for this application, lecithin was found to be a most effective and stable agent. Addition of lecithin to the concentrate before spray drying was ineffective. Coating with a dispersion of lecithin in water required a concentration on the powder of 0.6% phospholipid, whereas with lecithin applied in an oil or anhydrous milk fat carrier (phospholipid to oil ratio 1:2) a level of 0.2% was equally effective. Lecithins with hydrophilic properties enhanced by fractionation or special treatment have been found to give powders which exhibit very rapid wetting. Taste panel evaluations over a 12 month storage period demonstrated that the flavor of WMP treated with granular lecithin dissolved in anhydrous milk fat was indistinguishable from unlecithinated WMP.

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THE INDUSTRIAL USE OF SPECIAL LECITHINS. Willem van Nieuwenhuyzen, Unimills GmbH, Dammtorwall 15, D-2000 Hamburg 36, Fed. Rep. of Germany.

Soya lecithins are surface active substances which are used as excellent natural emulsifiers. The surface activity of the phospholipids can be adapted by fractionation or adjusted by enzymatic hydrolysis, acetylation and hydroxylation. Tailor-made lecithin specialities are important additives in the manufacturing of food, feed, pharmaceutical and chemo-technical products. Lecithin fractions and hydrolyzed lecithins have unique influence on the spattering behavior of margarine. Agglomeration processes for the production of instant foods require the use of refined lecithins with adjusted phospholipid composition. For many years lecithins have been used in technical industries such as the paints industry. New application areas such as mosquito control systems and leather dubbing are opportunities to extend the use of lecithins. The fundamental principles of these applications will be presented.

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THE DETERMINATION OF VITAMIN D IN MARGARINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. Pieter J van Niekerk and Salomien C.C. Smit, National Food Research Institute, CSIR, P.O. Box 395, Pretoria, 0001, South Africa.

Margarine manufactured in South Africa contains vitamin D added at a level of one to three international units per gram. Due to the high ratio of lipid to vitamin D, it is difficult to determine the vitamin for control purposes. A high performance liquid chromatographic method for the determination of vitamin D in margarine is proposed. The unsaponifiable fraction of a margarine sample is chromatographed on an adsorption system. The vitamin D fraction is collected and then rechromatographed on a second adsorption system having a different selectivity. The vitamin D fraction collected from this column is finally injected onto a reversed phase system where the vitamin D_2 and D_3 is separated and quantitated with an ultraviolet detector. Vitamin D_2 is used as an internal standard for vitamin D_3 or vice versa depending on the form of the vitamin added to the margarine. The second adsorption system is necessary to remove an interfering compound which co-chromatographs with vitamin D_2 on the reversed phase system.

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QUALITY CONSIDERATIONS MARGARINE OIL MANUFACTURING. Savinay Patel, Central Soya Company, Inc., Food Res. and Engr. Center, 1300 Ft. Wayne Bank Bldg., Ft. Wayne, IN 46802.

This presentation covers the critical areas of the manufacturing processes affecting the quality of margarine oils, from the source materials to the finished products. While major emphasis is placed on soybean oil-based margarine oils, the processing and handling of the more fragile oils, such as corn oil, are included. The process areas covered are: refining, bleaching, hydrogenation, blending. deodorization, storage, and handling. The advantages of process engineering automation, stainless steel equipment, inert gas or nitrogen blanketing to protect flavor and keeping quality are described. Quality Control checks, exercised to provide the best quality margarine oils to meet customer specifications, are highlighted. The importance of margarine oil specifications in context of manufacturing considerations are also discussed. Quality considerations involved in shipments of the bulk margarine oils are discussed in reference to energy and cost savings.

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GRAS CHEMICALS AS AFFECTORS IN PRESERVATIVE SYS-

TEMS. Jon J. Kabara, Dept. of Biomechanics, MSU/COM, East Lansing, MI 48824.

Because of new and stronger government regulations, the introduction of any new chemical preservative into the food chain is highly improbable, time consuming and costly. Unfortunately many of the approved food-grade preservatives do not adequately protect products under all conditions. Because of the energy crisis the preservation of foods by cold storage alone needs to be reevaluated. The shelf life of products needs to be extended and food cost due to such waste reduced. Rather than simply adding new or more old preservatives to the product, I advocate a new approach to the problem. The food system should be the preservative. An example of this "systems approach" is given by pointing to the new functionality of emulsifiers, antioxidants and chelating agents. Lauricidin (monoglyceride of lauric acid), tert. butyl hydroxy anisole (BHA) or tert. butylhydroxy toluene (BHT) and ethylenediaminetetroacetic acid (EDTA) have been shown simply and collectively to adversely affect the growth of organisms. These common GRAS chemicals are useful affectors whose toxicology and safety are well known. In addition, common food preservatives can be put into products at lower concentrations. The structure-function relationship and potential use of these chemicals as preservative affectors will be discussed.

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EVALUATION OF THE FUNGISTATIC PROPERTIES OF LAURICIDIN AND LAURICIDIN PLUS. Ahmad Moustafa, and James R. Agin, The Miami Margarine Co., 5226 Vine St., Cincinnati, OH 45217.

Previous work has been conducted to evaluate the bacteriostatic and fungistatic properties of lauricidin (monoglyceride of lauric acid) and lauricidin plus employing liquid media. The purpose of this study was to develop a method of evaluation on semi-solid media, and to determine inhibitory concentrations of the test materials against *Aspergillus niger* ATCC No. 16404, *Penicillium funiculosum* ATCC No. 11797, *Cadida albicans* ATCC No. 10271, *Bacillus subtitlis* ATCC No. 6633, *Pseudomonas aeroginosa* ATCC No. 9027, and *Staphylococcus aureus* ATCC No. 6538. The method employed consisted of incorporating the test material into an agar medium and inoculating the surface with the test material. Results indicated that a concentration of 180 ppm lauricidin exhibited stasis against most of the test organisms, while a greater concentration was required for lauricidin plus.

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A COMPARISON BETWEEN THE CONDITIONS OF MANUFACTURE FOR A HYDROGENATED FISH OIL MARGARINE AND FOR A VEGETABLE OIL MARGARINE. Charles H. Struble, Alvaro Del Solar and Jorge Gallo, CIA Industrial Peru Pacifico S.A., 5349 Plantation Road, Plantation, FL. 33317.

The work was done during the development of margarine manufacturing procedures in a new plant at a location where hydrogenated fish oil blends and hydrogenated vegetable oil blends were used. With adjustment of processing conditions and minimum of blending with available oils, standard house stocks of hydrogenated fish oils and, on the other hand, house stocks of hydrogenated palm-cottonseed blends produced acceptable, relatively low cost margarines. The differences between the margarine manufacturing procedures for the fish oil base margarine and the vegetable oil margarine will be discussed.

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RAPESEED ECONOMICS AND TRADE. Allan E. Earl, Winnipeg, Manitoba, R3B 1B3, Canada.

The production of rapeseed is being encouraged within the European Economic Community and has historically occurred on very large scale in Asia. However, while most producing nations remain in, at best, self sufficiency positions, Canada has become the largest producer (45% of the total) and certainly the most significant exporter. Within that country, rapeseed oil has rapidly displaced imported oils to become the chief component of the edible oil market place. Much of the domestic development has been achieved through considerable quality-based genetic engineering. The increased export market reflects a combination of these quality improvements and beneficial foreign exchange relationships. The latter (paradoxically) allow Canadian agri-business to achieve improved returns and importing nations to achieve better prices.

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SOY OIL—THE KING WITH NO CROWN. David R. Erickson, Donald G. Frahm, and Parry Dixon. American Soybean Association, 777 Craig Road/P.O. Box 27300, St. Louis, MO 63141.

The rapid growth of soy oil production and utilization to its present U.S. and world dominance will be reviewed. The reasons for this growth and expected continued dominance will be discussed. The phenomena of market dominance with relatively little consumer awareness will be shown and discussed as a unique situation in the marketplace. The concurrent development of appropriate technology in response to demand and availability of soybeans and soy oil will also be reviewed.

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THE U.S. SUNFLOWER INDUSTRY PRODUCTION AND MARKETING OUTLOOK. Allen A. Housh, Cargill, Inc., Box 9300, Minneapolis, MN 55440.

Cargill introduced oil sunflower to the Red River Valley farmer in 1966 to give the farmer an attractive cash crop alternative and to supply another oilseed for Minneapolis-based crushing plants to replace declining linseed oil demands. Strong world price levels for oilseeds coupled with genetic breakthroughs on sunflower seed yields and oil content make oil sunflower a highly competitive crop for land use. United States production in 1979 doubled over that of 1978, at the same time, Russian production decreased and world demand for vegetable oils increased. Presently 80 percent of U.S. sunflower seed is exported. Projected increases in U.S. crushing capacity for sunflower seed together with broader U.S. and export markets for the oil will tend to stabilize sunflower seed and oil markets.

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THE COTTONSEED INDUSTRY: ECONOMICS AND MARKETING. J. Allen Ater, P.O. Box 2538, Houston, Texas 77001.

Cottonseed oil production has been quite variable in the United States over the years, but has tended to stabilize in the range of 1.3 to 1.5 million pounds in recent years. Domestic usage has been in a long range decline but is leveling off at around 600 million pounds per year. More than 50% of domestic production has been exported in each of the past five marketing years, with Egypt, Venezuela, Iran, and Japan among the principal buyers. Other traditional customers, including West Europe, India, and Pakistan have virtually ceased importing cottonseed oil in favor of other oils. The greatest technical advance influencing cottonseed oil quality has been miscella refining, a technique that minimizes refining losses and color reversion. More oil each year is sold from first hands as once-refined, usually as Prime Bleachable Summer Yellow or Prime Summer Yellow. Nearly all oil exports are once-refined, a development that has dramatically reduced problems and disputes related to color reversion and free-fatty-acid build-up during storage and transit. Cottonseed oil is facing heavy competition in export markets. Sunoil production has been increasing rapidly and is competing hard for premium markets. At the same time, major importers are adopting new technology and experimenting with blends of lower priced oils in premium products. This will inevitably influence long range distribution patterns and price relationships for cottonseed oil.

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ECONOMIC AND MARKETING OPPORTUNITIES IN THE ANIMAL FATS INDUSTRIES. Werner R. Boehme, Fats and Proteins Research Foundation, Inc., 2720 Des Plaines Avenue, Des Plaines, IL 60018.

The demands in both domestic and foreign markets for animal fats, loosely called tallow, have changed dramatically since the late 1940s. At that time, the advent of synthetic surfactants derived from petrochemical sources brought about a rapid decline in the consumption of tallow for soap and forced the rendering industry to develop new markets for these annually renewable resources. Today, the animal feed industry is the largest single consumer of tallow with fatty acids and their derivatives in second place. In recent years a reversal in the declining use of tallow by the soap industry is becoming apparent. The rapidly spiraling cost of petroleum, current trends in the livestock and meat-packing industries, and the substitutability of vegetable glycerides are among the factors responsible for the changing usages of tallow which appear to follow the classical rules of derived demand in economics.

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"DIREX" APPLICATION OF THE DIRECT SOLVENT EXTRACTION SYSTEM ON VARIOUS HIGH OIL CONTENT SEEDS WITH PARTICULAR REFERENCE TO WET-MILLED CORN GERM. E. Bernardini and M. Bernardini,* Costruzioni Meccaniche Bernardini C.M.B., S.p.A., Via Della Petronella, 00040 Pomezia (Roma), Italy.

This paper depicts the "DIREX" system which enables high oil-bearing seeds to be directly extracted without the use of continuous screwpresses. A series of comparative tables show the advantages of the "DIREX" system vs. other extraction procedures. The DIREX system has been successfully applied on wet-degerminated corn germ containing up to 50% oil. Mention is also made of actual operating results and data obtained from tests carried out on an industrial scale by one of the most important corn processors in the world who has recently adopted the DIREX system.

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DEHULLING OF OILSEEDS. F.W. Sosulski and R. Zadernowski, Department of Crop Science, University of Saskatchewan, Saskatoon, Sask. Canada S7N 0W0.

The removal of hulls is necessary in the preparation of protein-rich fractions for food uses. There are mechanical problems and disadvantages in dehulling prior to oil extraction of sunflower and canola seeds. Therefore, techniques have been devised for concurrent oil extraction and dehulling or hull removal after desolventization. Data are presented on the optimization of the process, yields of products and their compositional characteristics.

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A NEW METHOD OF DETOXIFICATION OF COTTONSEED BY MEANS OF MIXED SOLVENT EXTRACTION. Fu-Kuang Liu, Shyh-Yin Jou and Li-Yuh Jung, Light Industries Institute, Wushi, Kiangsu, Peoples Republic of China.

For several decades scientists in the field of vegetable oil have been trying to detoxify cottonseed, but up to now they haven't found a practical method. We have found that using 20–30% by weight of ethyl alcohol (90% in volume) with commercial hexane as a mixed solvent to extract either cottonseed prepressed cake or flake, both gossypol and oil can be effectively extracted at the same time. The result is free gossypol is reduced to about 0.013–0.04%– the total gossypol being reduced to about 0.32–0.55%, while the residual oil is reduced to about 0.5% or even less. The aflatoxin, if any, will also be eliminated in the process. The detoxified cottonseed meal can therefore be used as animal feeds and the resultant cottonseed protein can be used as a substitute, partially or wholly, for soybean protein. The protein crisis felt in the world today might be alleviated to some extent using this method. The oil thus extracted will have a better quality than that obtained by the usual hexane extraction method.

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FRACTIONATION/WINTERIZING OF EDIBLE OILS. Peter C. Linnemann, Neumunz, Inc. 117 Fort Lee Rd., Leonia, NJ 07605.

A review of current practices of fractionation and/or winterization as it applied to palm oil for the formation and separation of liquid fraction from solid fraction or stearine is given. Different methods, crystallization and phase separation, including fractionation in miscella phase will be discussed.

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PILOT PLANT DESOLVENTIZING-TOASTING OF EXTRACTED SOYBEAN FLAKES–A PRELIMINARY STUDY. K.J. Moulton, G.C. Mustakas* and E.C. Baker, SEA/AR/NRRC/USDA, 1815 North University Street, Peoria, IL 61604.

A pilot plant batch desolventizer-toaster (D-T) was designed and built with the capability of simulating a continuous commercial D-T unit, except that a batch-sequence procedure was used. Dry runs were made first to determine optimal loading levels, temperature control, and steam generation operation for sparge steam. After-sparge moisture levels were influenced by the residual hexane content of spent flakes reaching the D-T. Two moisture levels, as determined by residual hexane content, were used to study the toasting operation. Conditions for toasting soybean meals to low-urease activity and trypsin inhibitor levels were determined. The shakedown runs reported here will provide basic data to be used in future work seeking to optimize toasting procedures for animal nutrition studies.

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OVERALL TRAY EFFICIENCY IN CONTINUOUS EDIBLE OIL DEODORIZERS. Anacleto González Flores and Ramon Sanz Castaños, Desarrollo Industrial Ings. S.A., Casma #515, México 14 D. F.

A revision of the most well-known correlations for predicting overall tray efficiency in bubble cap tray and similar tray continuous deodorizers built like continuous distillation columns is presented. The revised conversion of Murphee efficiency to overall plate efficiency, and the AIChE method for prediction of overall efficiencies in distillation columns is discussed. Commercial overall tray efficiencies in continuous deodorizers using valve-type trays and bubble cap trays are discussed. The lack of experimental data on solubilities of volatiles in oil to predict equilibrium constants is mentioned, and comments on contacting devices used in European-type deodorizers which do not use petroleum contacting internals are also presented. Prediction of the actual number of stages required for vegetable oil continuous deodorizing relies on sound theory. However, data on solubilities of oleic and other fatty acids in oils are not readily available in known publications for determining K.

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WIPED FILM EVAPORATOR PURIFICATION OF FATTY ACIDS FROM ACIDULATED SOAPSTOCKS. G.V. DeLaney, The Pfaudler Co., Div. of Sybron Corporation, P.O. Box 1600, Rochester, NY 14603.

The Wiped Film Evaporator can be used to obtain good clean low color fatty acid mixtures from the associated acidulated soapstock. The unique properties of the wiped film evaporator including high vacuum and very short residence time make this a very facile purification technique. As more companies are looking to further utilize their byproducts–the wiped film evaporator offers a technique to consider.

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PROCESSING AND PACKAGING CONSUMER PRODUCTS AT THE OIL REFINERY. Ray Edmunds and Frank Khym, Edmunds Machine Company, P.O. Box 17526, San Antonio, TX 78217.

A discussion is given of methods and equipment available for small oil refineries to meet the growing need of fat and oil products in consumer size packages. Procedures for testing product components, formulation, control during the processing-packaging operations and storage of the finished products will be emphasized. Major finished products to be considered are salad and cooking oils; liquid and solid shortenings for frying, baking and general purpose use; table grade and pastry margarines. Reference to consumer size packages in this discourse includes retail for family consumption and sizes for institutional food service and commercial bakeries.

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ENVIRONMENTAL ASPECTS OF SOLVENT EXTRACTION. Noel W. Myers, Myers Engineers, P.O. Box 1493, Decatur, IL 62525

The Federal EPA is engaged in adopting regulations to reduce to extremely low levels the allowable emissions of hexane in the soybean extraction industry. This paper will bring an up-to-date report on the activity of the EPA in pursuing what is thought to be unrealistic limits with their proposed processing techniques that have inherent fire and explosion risks as well as large capital requirements. The author is the environmental engineering consultant for the National Soybean Processors Association and is involved in this sensitive area.

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INFLUENCE OF *TRANS*-FATTY ACIDS ON THE METABOLISM OF *CIS* AND SATURATED ACIDS IN DEVELOPING BRAIN. Harold W. Cook, Departments of Pediatrics and Biochemistry, Atlantic Research Centre for Mental Retardation, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Trans-unsaturated fatty acids, formed by partial hydrogenation of unsaturated acids during commercial processing of oils or by rumen microorganisms, can be significant dietary components and are incorporated into complex lipids of many tissues. We have shown that *trans* acids can constitute up to 3% of the total octadecenoic acyl chains of human brain lipids. Also, *trans* acids cross the blood-brain-barrier and are rapidly incorporated into the complex lipids of developing rat brain; in contrast to *cis* and saturated acids, they are not active substrates for desaturation or chain elongation by brain enzymes. To investigate whether *trans*-unsaturated acids may be inhibitory to the metabolism of more abundant and metabolically active acids we have used two experimental models. In the first unlabeled acid [oleic (c-18:1), elaidic (t-18:1), linoleic (c,c-18:2), linoelaidic (t,t-18:2), arachidonic (20:4) and stearic (18:0)] was injected simultaneously with 60 nm (3 μCi) of [1-^{14}C] c,c-18:2 into the brain of 10-day-old rats. After 24 hr in vivo, little alteration of ^{14}C-18:2 metabolism and incorporation was seen with a 2-fold excess of competitor acid. In 15-fold molar excess, c-18:1 inhibited metabolism of 18:2 and incorporation of products into phospholipid, whereas t,t-18:2 and 20:4 had little effect and t-18:1 and 18:0 were stimulatory. The slight alterations appeared to be generalized and not directed at specific steps in the desaturation-elongation sequence. In other experiments, effects of various fatty acids on Δ9- and Δ6-desaturation and on chain elongation activitivities were measured using in vitro assays and rat brain preparations. Although dienoic isomers were more inhibitory than monoenoic, little difference between *cis* and *trans* isomers of equivalent unsaturation was observed. Thus, *trans*-acids are no greater as inhibitors of fatty acid conversions than are their *cis* isomers. Accordingly, *trans*-acids are unlikely to be deleterious as specific inhibitors of metabolic pathways leading to acyl chain modification and incorporation into membrane lipids in the central nervous system, even if present in relatively high concentrations.

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PLACENTAL TRANSPORT OF TRANS FATTY ACIDS IN RATS. Carolyn E. Moore and Govind A. Dhopeshwarkar, Laboratory of Nuclear Medicine and Radiation Biology, UCLA, 900 Veteran Ave., Los Angeles, CA 90024.

Transport of elaidic (18:1t) and linoelaidic (18:2t,t) acids was demonstrated from maternal plasma to placental and fetal tissues in rats. On the 18th day of gestation, a ^{14}C-labeled albumin complex of elaidic or linoelaidic acid was injected into the maternal circula-

tion. All animals were sacrificed 1 hr after injection. Lipid composition and distribution of label were determined in placenta, fetal tissues and maternal plasma. Differences in specific activities of plasma, placental and fetal total lipids suggested that a maternal-fetal concentration gradient of elaidic and linoelaidic acids exists. Specific activity (cpm/mg) of maternal plasma total lipids was 4 to 7 times greater than specific activity of placental total lipids, which was several fold greater than specific activity of fetal total lipids. Distribution of radioactivity in various lipid components was determined by TLC. Irrespective of the label, the highest percentage of total radioactivity was carried by the triglyceride fraction in maternal plasma (∿60–80%) and was incorporated primarily in phospholipids of fetal tissue (∿50–60%). A nearly equal distribution of the label was found between phospholipids and triglyceride fractions of placental lipids (∿40%). Radioactivity of fatty acid methyl esters (FAMES) determined by Radio-GLC indicated that after injection of linoelaidate, radioactivity of maternal plasma, placenta and fetal tissue FAMES was associated only with 18:2 t,t. Following injection of elaidate, all radioactivity in placental FAMES was associated with 18:1t. However, the label in fetal tissues was distributed between 16:0 and 18:1t. These findings suggest that by the 18th day of gestation, rat fetal tissue can metabolize elaidic acid via β-oxidation.

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INCORPORATION OF POSITIONAL ISOMERS OF DIETARY *CIS*- AND *TRANS*-OCTADECENOIC ACIDS INTO TISSUE LIPIDS OF THE RAT. Kumar D. Mukherjee, Federal Center for Lipid Research, Piusallee 68, D-4400 Münster, Germany.

In rats fed partially hydrogenated soybean oil, the pattern of incorporation of dietary *cis*- and *trans*-octadecenoic acids was found to be specific for the different tissues. Most of the *cis*- and *trans*-octadecenoic acids were found in adipose tissue, adrenals and testes in proportions similar to those in dietary triacylglycerols, whereas several *trans*-octadecenoic acids were enriched in other tissues. Thus, each of the *trans*-12- through *trans*-15-octadecenoic acids was preferentially incorporated into liver, heart and serum, while the *trans*-10- and *trans*-11-isomers were distinctly excluded from these tissues. The following patterns of incorporation were found in the major lipid classes of liver, heart and serum. *cis*-9-Octadecenoic acid was selectively esterified in cholesteryl esters of liver and serum, wheras *trans*-9-octadecenoic acid was selectively incorporated into cholesteryl esters of heart. In the triacylglycerols of the three tissues, *cis*-9-octadecenoic acid was preferentially esterified at position 2, while each of the *trans*-11- through *trans*-15-octadecenoic acids was preferentially located at positions 1,3. Relatively little *cis*-9- and *cis*-11-octadecenoic acids, or correspondingly large proportions of *trans*-9- and each of the *trans*-11- through *trans*-15-octadecenoic acids were incorporated into diacylglycerophosphocholines of each tissue, whereas the *trans*-10-octadecenoic acid was distinctly excluded from these lipids. In the diacylglycerophosphocholines, both *cis*-9- and *cis*-11-octadecenoic acids were selectively esterified at position 2 and each of the *trans*-octadecenoic acids was selectively incorporated into position 1. Abundance of *trans*-octadecenoyl moieties at position 1 of diacylglycerophosphocholines, as compared to positions 1,3 of triacylglycerols, suggests that within the glycerophosphate pathway, the 1,2-diacylglycerols, containing *trans*-octadecenoyl moieties at position 1, are selectively used for the synthesis of diacylglycerophosphocholines rather than triacylglycerols.

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EFFECT OF A *TRANS* FATTY ACID (ELAICID ACID) ON THE THERMOTROPIC PROPERTIES OF A BACTERIAL MEMBRANE AND ITS LIPID COMPONENTS. J.D. Morrisett, H.J. Pownall, R.T. Plumlee, L.C. Smith, R.D. Yang, K.M. Patel, R.D. Knapp, L.A. Sklar, R.B. Crawford, Z.E. Zehner, M. Esfahani and S.J. Wakil, Baylor College of Medicine, Houston, TX 77030.

At characteristic temperatures, membranes from *Escherichia coli* cells enriched in exogenous elaidic acid exhibit two abrupt changes in the slope of Arrhenius plots for two enzyme activities. For NADH oxidase, these changes occur at 27 C and 32 C, whereas for D-lactate oxidase, these changes occur at 31 C and 36 C. Pyrene excimer fluorescence and spin-labeled fatty acid paramagnetic resonance results indicate that the beginning, midpoint, and end of a single structural change (order→disorder transistion) occurs at 25.5-29.0 C, 30.0–31.0 C, and 33.0–35.5 C, respectively. These data suggest that for NADH oxidase, the observed activity changes correspond to the beginning and midpoint of a single membrane lipid structural change, whereas for D-lactate, the activity changes correspond to the midpoint and end of that structural change. In addition to the membrane structural change spanning the range of 25.5–35.5 C, a second change (9.5–21.0 C) was observed. This transition was detected by 5- and 16-2,2-dimethyl-oxazolindinyl-l-oxyl (doxyl) stearates, but not by 12-doxyl stearate or pyrene. Structural changes in the extracted lipids were observed in the temperature ranges 4.0–9.0 C, 14.0–20.0 C, and 25.0–35.5 C. The two higher ranges correlate well with the ranges for structural changes observed in the intact membrane. Observations of these multiple transitions in both intact membranes and extracted lipids strongly suggest that these lipids segregate into domains of different fluidity and composition. Dielaidoylphosphatidylethanolamine, the principal lipid component of these membranes has been studied by paramagnetic resonance, fluorescence, and calorimetric methods. EPR measurements with perdeutero-di-*tert*-butylnitroxide and 2,2,6,6-tetramethyl piperidine-1-oxyl indicate that, when dispersed in aqueous media, this phospholipid undergoes an abrupt order→disorder transition at 37.5 C and 36.5 C, respectively. A similar transition temperature is suggested by experiments with 9-doxyl-dimyristoylphosphatidylethanolamine (DEPE). *cis*- and *trans*-Parinaric acid fluorescence polarization measurements indicate that the midpoint of this transition occurs at 34.0 C and 35.5 C, respectively. Differential scanning calorimetry of DEPE revealed a single, sharp endotherm at 38.5 C with increasing temperature; two exotherms of similar magnitude were observed at 36.5 C upon cooling. This double transition was not observed by any of the other methods. From these results we conclude that the major structural transition at 30–31 C observed with 5-, 12-, and 16-doxyl stearate in intact *E. coli* membranes is due to the DEPE present.

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INCORPORATION OF ISOMERIC FATTY ACIDS IN EGG AND HUMAN BLOOD LIPIDS. Herbert J. Dutton, Northern Regional Research Center, AR,SEA,USDA, 1815 North University, Peoria, IL 61604.

Use of dual isotopic labels in experiments with the laying hen and with humans permits comparison of incorporation for two isomeric fatty acids under essentially identical conditions. This design enhances the precision of results. One fatty acid, usually oleic, is arranged to be common in each of a series of experiments so that intercomparison of all isomers tested is possible. Because of relative convenience of synthesis and analysis, radioactive isomers tagged with ^{14}C and ^{3}H are frequently used in in vitro and in laying hen experiments. Deuterium-tagged fatty acids are used with humans for safety reasons. Triple-label experiments are possible, using fatty acids tagged with different numbers of deuterium atoms. Because of the relative speed of radioactive experiments, the hen frequently serves as pilot for human studies with stable isotopes. Thus, laying hen experiments have been reported using isomers of oleic acid with *cis* double bonds in the 8, 10, 11 and 12 positions, of oleic with elaidic acid; and of linoleic with linoelaidic acid. Distribution of these isomers has been followed in the various egg yolk lipids. In humans, the incorporation has been reported of oleate and elaidate in dual-label experiments and of *cis*- and *trans*-12-octadecenoate with oleate in triple-label experiments. The distribution of these isomers in neutral lipids and phospholipids of plasma, erythrocytes and platelets is reviewed and summarized, together with the extensive list of isomers studied in the hen.

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EFFECTS OF *TRANS* LINOLEATE ON TISSUE LIPIDS AND SERUM PROSTAGLANDIN CONCENTRATIONS. J.E.Kinsella, J.L. Shimp and J. Mai. Cornell University, Department of Food Science, Stocking Hall, Ithaca, NY 14853.

Feeding high concentrations of *trans,trans*-9,12-linoleate to rats (i.e. at 50 and 100% of dietary fat) depressed growth rates and heart, kidney and lung weights. The concentrations of n6 fatty acids 18:2, 20:3 and 20:4 in heart, lung, kidney, liver and platelets were decreased. *Trans* acids did not accumulate to a significant extent in any of the organs and the level of 20:3n9 was not as high in rats on *trans,trans*-18:2 as that usually observed in rats on diets deficient in essential fatty acids. In conjunction with the depression of 20:3n6 and 20:4n6 in platelets and serum a significant decrease in the quantities of serum prostaglandins (PG) was observed. Thus, the concentrations (ng/ml) in rats receiving all *cis,cis*-18:2 and all *trans,trans*-18:2 was 5.7 and 0.2 for PGE_1; 24.9 and 0.1 for PGE_2 and 27.4 and 0.30 for $PGF_{2\alpha}$ respectively. The *trans,trans*-18:2 inhibited acyldesaturases and thereby may have imparied PG production.

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EFFECT OF *TRANS* ACIDS ON THE METABOLISM OF LINOLEIC ACID IN THE RAT AND HUMAN. Robert L. Anderson, Procter & Gamble Company, Miami Valley Laboratories, P.O. Box 39175, Cincinnati, OH 45247.

Ingestion of high levels (>5% of diet acids) of the di-*trans* isomer of linoleic acid (18:2) inhibits the conversion of 18:2 to 20:4 in liver lipids but does not act as a substrate for *trans*-20:4. In contrast, the mono-*trans* linoleate isomers do not inhibit the 18:2→20:4 reaction and can act as a substrate for *trans*-20:4 synthesis. Similar effects of the *trans* isomers of linoleate were noted in the testis lipid but all of the effects were less pronounced than those seen in the liver. In contrast to the effects of the *trans* isomers of linoleate noted in rats, humans fed a diet designed to provide ∿100 g of

hydrogenated oil (∿20 g of *trans* acids) intake per day for 7 days showed an increased *trans* acid content but no evidence of diminished 18:2→20:4 conversion as assessed by plasma phospholipid fatty acid compositions. This suggests that the *trans* acids in hydrogenated oils (mostly *trans* 18:1) do not have the same inhibitory effects on 18:2→20:4 as has been demonstrated for the *trans* isomers of linoleic acid.

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EFFECT OF DIETARY *TRANS* FATTY ACIDS ON MEMBRANE LIPIDS: THE FATTY ACID COMPOSITION OF LIPIDS OF BRAIN AND LIVER MITOCHONDRIA AT BIRTH AND DURING POST NATAL DEVELOPMENT. Jan Petterson and Johannes Opstvedt, Norweigian Herring Oil and Meal Industry Research Institute, N-5033 Fyllingsdalen-Bergen-Norway.

The objectives of the investigation were to study the effects of dietary *trans* fatty acids on the fatty acid composition of membrane lipids with special emphasis to brain and liver mitochondria lipids. Female pigs were fed diets containing 4% of sunflowerseed oil plus 14% of experimental fats from 3 weeks old till 3 weeks after parturition. The experimental fats were partially hydrogenated fish oil, partially hydrogenated soybean oil and lard. The pigs were bred to farrow at an age of about one year. Half of each littler were sacrificed immediately after parturition, the other half after three weeks of suckling at which time the mothers also were killed. Samples were obtained from brain, liver, heart, and adipose subcutaneous tissue and milk. Total lipids were extracted by chloroform-methanol, and lipid classes separated on TLC. Fatty acid composition was determined by GLC. Geometrical *(cis and trans)* and position isomers of monoenes were separated by capillary glass column GLC, and total *trans* fatty acids were determined by IR spectrophotometry. Effects on membrane lipids will be presented and discussed in relation to placental and mammary transmission of *trans* fatty acids of different chain lengths.

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THE ATHEROGENIC EFFECT OF *TRANS* AND SATURATED FATTY ACIDS IN RABBITS. J.J. Gottenbos and R.O. Vles, Unilever Research, Vlaardingen, P.O. Box 114, 3130 AC, Vlaardingen, The Netherland.

It can be concluded from the studies on the metabolism of *trans* fatty acids that the *trans* monoenoic fatty acids behave like *cis* monoenoic fatty acids or saturated fatty acids depending on the physiological criteria selected. Effects of linoleic acid isomers on cell membrane functions are observed only in the absence of linoleic acid; this can be explained by the preferential incorporation of dietary linoleic acid and its metabolite arachidonic acid into membrane phospholipids. In nutritional experiments, the atherogenicity of *trans* fatty acids was compared with that of saturated fatty acids. Rabbits were fed cholesterol-free diets containing 40% fat. The basic fat mixture contained 30% saturated fatty acids and 10% linoleic acid. To this mixture 20, 30 or 40% of either saturated or *trans* fatty acids were added, the rest being oleic acid. After 2 years, aorta atherosclerosis was scored. No significant differences in atherogenicity between *trans* or saturated fatty acids were found. Furthermore, it was demonstrated that the atherogenic effect of *trans* fatty acids is reduced by linoleic acid as is the atherogenicity of saturated fatty acids. It is concluded that dietary *trans* fatty acids are equally well tolerated as saturated fatty acids, provided the diet contains sufficient linoleic acid.

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FACTOR AFFECTING WAX DETERMINATION AND REMOVAL FROM SUNFLOWERSEED OIL. W.H. Morrison III and J.A. Robertson, USDA, SEA, Russell Research Center, Field Crops Laboratory, P.O. Box 5677, Athens, GA 30604.

Wax removal or winterization has become an even more interesting problem with the greater use of sunflowerseed oil by the consumer. This report will deal with some of the constituents in the oil that affect winterization and an evaluation of a method for determining wax content.

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SUNFLOWER SEED PROCESSING. Glenn D. Brueske, Crown Iron Works Company, 1229 Tyler Street, N.E., P.O. Box 1364, Minneapolis, MN 55440.

Although sunflowers have been processed for many years in Europe, they are a relatively new oilseed to North America. They are rapidly increasing in production and processing capacity is expanding. Emphasis will be placed on the pre-press solvent extractor method of processing. Various techniques in preparation of seed as used in present and proposed oil mills will be discussed. A brief discussion on dehulling will be given with the pros and cons resulting from the dehulling step. Slides of various process flows, pictures of pre-press expellers and solvent extraction equipment will be shown.

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SUNFLOWER OIL PROCESSING FROM CRUDE TO SALAD OIL. Frank E. Sullivan, Sullivan Systems Inc., P.O. Box 158, Tiburon, CA 94920.

While world-wide consumption of sunflower oil is second to soybean oil, interest in domestic use as a premium salad oil is recent. The high ratio of polyunsaturated to saturated fatty acids makes sunflower oil a premium product as a salad oil. Sunflower oil, however, contains a small amount of high melting wax which must be removed to avoid settling problems when packaging. It is possible to produce a brilliant, dewaxed, deodorized sunflower oil that withstands a 100-hour cold test at 0 C. This quality oil may be produced by conventional caustic refining, dewaxing, bleaching and deodorization. A quality finished oil may also be produced by dewaxing and steam refining. This paper will review various methods for producing sunflower oil from the crude state to finished salad oil.

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MODERN PROCESSING OF SUNFLOWERSEED. H.L.S. Staff, H.L.S. Ltd., P.O. Box 193, Petah-Tikva, Israel.

The trend to increase the growing of sunflowerseeds requires using up-to-date technology for oil processing. In many countries, factories process sunflowerseed without dehulling. The disadvantages are: meal with low protein content, more wear and tear of equipment and higher energy consumption. A description is given of dehulling systems used in Eastern Europe and a new approach to dehulling equipment with bigger capacities. The use of hulls for producing steam and energy-saving or furfural production and the feasibility of these uses are discussed. Comparison between direct extraction and prepressing plus extraction have advantages and disadvantages. Extraction equipment is presented. Theoretical processes for winterizing sunflowerseed oil and continuous winterization plants are recent developments in this field. Advanced methods for refining sunflowerseed oil (physical refining) are explained.

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SUNFLOWER DEHULLING TODAY. Willi Fetzer and Jack A. Hostettler,* Buhler-Miag, Inc., P.O. Box 9497, Minneapolis, MN 55440.

Over the last decade, sunflower seed has dramatically gained in importance and has become a major oilseed crop in South America and the USA. As a result, plant sizes for newly built sunflower processing facilities have steadily increased. The layout for a sunflower dehulling plant has undergone a steady movement to cope with these new requirements. The layout for the dehulling plant is discussed in regard to single and multiseed plants, considering reliability and maintenance aspects.

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RECENT DEVELOPMENTS IN SUNFLOWER PROTEIN TECHNOLOGY. F. Sosulski, Department of Crop Science, University of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0.

The presence of hull material and chlorogenic acid represents the principal constraint to the use of sunflower protein in food products. Plant breeding for white-hulled cultivars and low chlorogenic acid content in the seed are potential procedures for overcoming the problems associated with flour color. Aqueous diffusion of phenolic compounds can be achieved by short time-high temperature procedures which minimize protein denaturation. The removal of color-forming compounds with acidic butanol results in protein concentrates and isolates which have high nitrogen solubility, fat absorption and emulsification characteristics. Succinylated sunflower proteins show improved oil retention and foaming properties but water absorption was not improved. The potential of sunflowers as a source of low-methoxyl pectin and dietary fiber will be discussed.

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SUNFLOWER SEED DEHULLING SYSTEMS. George M. Nuemunz, Neumunz, Inc. 117 Fort Lee Road, Leonia, NJ 07605.

Commercial sunflower seed dehulling systems will be discussed, including advantages and disadvantages. Presentation will include flow diagrams and equipment illustrations. Actual plant data will be discussed.

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STUDY OF THE NEUTRAL LIPIDS OF SUNFLOWER ISOLATES. Francisco Millán, Eduardo Vioque* and Ma.Pilar Maza, Instituto de la Grasa, Sevilla, Span.

Two types of sunflower isolates have been obtained from press and solvent extracted sunflower meal. The first is obtained by precipitation at the isoelectric point of the alkaline extract of the meal and then washing the curd with water. The second isolate was obtained as before but the curd was washed with water, ethanol and acetone. Both isolates were first air dried and then under vacuum at

50 C. The neutral lipids associated with the proteins were extracted from the total lipids with 86% ethanol, using a column of Florisil. The lipid studied include those from the two types of isolates mentioned and also the lipids from the original meal for comparison. The following types of compounds were separated, identified and quantified: waxes, methyl esters, triglycerides, free fatty acids, free sterols, diglycerides and hydroxy-fatty acids. Several physical and chemical methods were used for the qualitative and quantitative study of the different components.

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RECENT DEVELOPMENTS IN THE PRODUCTION OF SUNFLOWER AND SUNFLOWER OIL IN HUNGARY. J. Holló, Department of Agricultural Chemical Technology, University of Technical Sciences, Budapest, H-1521 Budapest, Gellért tér 4, E. Kurucz, National Enterprise for Vegetable Oil and Detergent Industry, and J. Perédi, Research Institute for Vegetable Oil and Detergent Industry.

Hungary has traditions in proucing sunflowers and processing sunflower oil. Previously, only domestic varieties had been produced until the introduction of other high-oil content varieties–mainly from the Soviet Union–approximately 20 years ago. This contribution summarizes recent results in production (new Hungarian varieties, hybrids) and research investigations, which required the introduction of new processing technologies in storing, dehulling, extraction, winterization and oxidation properties. The paper gives a report on the development of vegetable oil production in Hungary and describes a new high-capacity vegetable oil plant in Martfű. Problems are outlined involving further efforts and tasks such as utilization of by-products, with special regard to dehulling residues.

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FRYING PERFORAMNCE OF PALM OIL LIQUID FRACTIONS. U. Bracco, A. Dieffenbacher and L. Kolarovic, Nestle Products Technical Assistance Co., Ltd. (NESTEC), Res. Dept., P.O. Box 88, CH-1814 La Tour-De-Peilz, Switzerland.

Palm oil liquid fractions were used as frying media in household and industrial fryers and compared to standard edible oils and fats, such as soybean, groundnut, sunflower, rapeseed and tallow. The analytical evaluation covered ffa, viscosity, smoke and flame points, oxidized fatty acids, nonelution material (NEM), UV differential spectra, polymers and "foam index". These figures allow judgement for the extent of the oil degradations, namely oxidation, hydrolysis, cracking and polymerization. These were combined with other analytical procedures (fatty acid composition, keeping the sample in a closed system during the time necessary for oxygen absorbing sample to reach a –0.5 psi pressure) in order to have a large analytical control during the frying processes. The data collected were analyzed for the suitability of edible oils and fats for frying purposes and indicated that palm oil liquid fractions perform satisfactorily as frying medium, show less extent of degradation and result in fried goods with acceptable keeping qualities.

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SAMPLING OF PALM OIL. D. Jacqmain, C.E.R.I.A. Avenue Emile Gryson, 1, B-1070, Brussels, Belgium.

The author has been in contact with sampling problems in the palm oil industry and developed a computation method which optimizes the number of samples to be taken in an inspection procedure. Basis for this method is that the levels of the probabilities for errors of the first and second kinds are not optimal values: they serve to initiate repeatable method. These probabilities must be determined in order to set up the sampling plan, i.e., the operating characteristic curve and the acceptance or refusal criterion. With a view to optimize the sampling size one has to take in account the sampling charges and the risks encountered. Some examples of problems specific to the palm oil industry are cited showing the difficulties met in the application of the method.

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EXTRACTION AND FRACTIONATION OF PALM OIL. Dato' B.Bek-Nielsen and S. Krishnan, United Plantations Berhad, Jendarata Estate, Teluk Anson, Perak, Malaysia.

The co-authors will present an appraisal of the research work by United Plantations Berhad leading to the development of a new screw press designed to produce high quality palm oil. Emphasis will be placed on the advantage of using stainless steel tanks, pumps and pipe lines, as well as the thermal treatment of crude and refined palm oil. Various aspects of fractionation by the Tirtiaux method will be analyzed and described by tables and curves which will be of interest to consumers of fractionated products.

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A COMBINED FRACTIONATION PROCESS FOR PALM OIL. K.G. Berger and B.K. Tan, Palm Oil Research Institute, 18th Floor, Angkasa Raya, Jalan Ampang, Kuala Lumpur 04-06, Malaysia, R.J. Hamilton, Liverpool Polytechnique, England, and B. Jacobsberg, Tropical Product Sales, Brussels.

A "dry-wet" double fractionation process was used for palm oil. Palm olein and stearin obtained by a detergent process were further fractionated using N-Hexane. The fractions were characterised by G.L.C., Differential Scanning Calorimeter and wideline N.M.R. The olein produced a fraction suitable for chocolate and confectionery applications and a liquid fraction for use as a frying oil. The stearin yielded a hard fraction consisting mainly of tripalmitin and a soft fraction suitable for use in margarine and shortening.

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FRACTIONATION OF PALM OIL. Richard Kassabian, Anadik, Inc., 504-76 St., North Bergen, NJ 07047.

Existing commercial methods for the fractionation of palm oil will be reviewed. Using palm oil as an example, the distinction between the commonly accepted views on winterization and the real meaning of fractionation will be explored. Multiple fractions, produced in the treatment of palm oil and various other triglycerides, produce fats with specific characteristics. Commercially valuable fats, available from straightforward fractionation techniques, provide significant profit potential. Selection suitable solvents precludes the necessity for using very low temperatures in most cases, thereby substantially reducing the processing cost.

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THE COMPOSITION AND PHYSICAL BEHAVIOR OF INTERESTERIFIED FATS WITH SPECIAL REFERENCE TO PALM OIL. H. Kifli and F.D. Gunstone, Chemistry Department, University of St. Andrews, KY 16 9ST, Fife, Scotland, UK.

Standard techniques of investigating component glycerides have been applied to palm oil alone and mixed with other fats/oils before and after interesterification effected by sodium methoxide as a catalyst. Pancreatic lipolysis results indicate that randomization is complete. The presence of methyl esters formed during interesterification has been established. An attempt is made to correlate differences in glyceride composition and melting behavior.

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PALM OIL–ECONOMICS AND MARKETING. Robert E. Miller, SOC Oil Corporation, Berhead, Kuala Lumpur, Malaysia.

Not available at press time.

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COMBINED USE OF HPLC AND INFRARED DETECTION FOR THE QUALITATIVE EXAMINATION OF ANIMAL FATS AND VEGETABLE OILS. Norman A. Parris, Du Pont Company, Instrument Products Division, Experimental Station, Building 334, Wilmington, DE 19898.

Fats and oils containing mixed glycerides are frequently analyzed by chromatographic methods. Column liquid chromatography using hexane/diethyl ether mobile phase with silica packings is used to separate oils into glyceride classes, i.e., mono-, di-, and triglycerides. Gas chromatography is used to establish the fatty acid composition, normally after saponification of the oil or fat and subsequent formation of the methyl esters. A reversed phase HPLC method has been developed which enables complex glyceride mixtures to be separated, without prior hydrolysis, using Zorbax™ ODS packing, a microparticulate silica packing with a high density of octadecyl bonded phase. It has been possible to resolve complex triglyceride sample mixtures by using mobile phases prepared from methylene chloride, acetonitrile and tetrahydrofuran. These solvents not only provide excellent solubility for the glycerides, thus avoiding sample overload conditions, but also can be used for gradient elution separations of glycerides that are monitored by infrared absorbance. The resultant chromatographic system, which is demonstrated to work best at elevated temperatures, is capable of directly separating many natural fats and oils. The operating wavelength of the infrared detector can be adjusted to impart selectivity to the system. The talk will be illustrated with examples, including the separation of components in soybean oil, castor oil, corn oil, and linseed oil.

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PURIFICATION OF LIPIDS ON A 100 mg. SCALE BY PREPARATIVE HPLC. W.S.M. Geurts van Kessel, Rijksuniversiteit Utrecht, Laboratory of Biochemistry, P.O. Box 80.054, 3508 TB Utrecht, The Netherlands.

The recent development of preparative high performance liquid chromatograph (PHPLC) has shown this technique has great possibilities for separation of phospholipid mixtures on a 100 mg to gram scale. Purification of 1,2-dioleoyl-sn-glycero-3-phosphocholine and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine from its reaction mixtures has been resolved. The lipid separation occurs on a Polygosil column and the individual compounds are monitored directly by refractive index detection. Chloroform-methanol-water and hexane-isopropanol-water mixtures are used as eluent systems,

providing a wide polarity range to separate all classes of lipids. The developed equipment can be used for columns of any size between 10 to 50 cm long, 4 to 50 mm inner diameter by any flowrate between 1 and 400 ml/minute and applied pressures between 10 and 450 bars.

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APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF THE GLYCERIDE COMPOSITION OF FATS AND OILS. A. Karleskind and M. Blanc, Laboratories WOLFF, 198, rue Sigmund Freud 75019 Paris, France.

In the field of routine analysis, the composition of oils and fats is known by the composition of fatty acids and sterols. However, most of the properties of oils and fats, and most of the industrial treatments which modify their characteristics, are based on the glyceridic composition and imply its knowledge. Gas chromatography enables one to determine the glyceridic composition by molecular weight only. Although high pressure liquid chromatography has been recently developeddue to improved equipment and porous packings, the direct analysis of the glycerides of oils and fats has not been done as a matter of routine: the great similarity, the multiplicity and the absence of characteristics of the glycerides make their detection very difficult. The quantitative halogenation of the unsaturated fatty acids of the glycerides, according to the principle of the iodine value, modify their polarities and give them a UV spectral characteristic. This makes their separation and detection by HPLC (with a UV detector) possible. This method allows a qualitative and quantitative analysis of the glycerides and permits one to determine the glyceridic composition of oils and fats.

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THE TRACE DETERMINATION OF FATTY ACIDS IN SMALL BIOLOGICAL SAMPLES AFTER FLUORESCENCE LABELING WITH Br-Mmc. W. Dünges and B. Soltau, Institut f.Biochemie, Deutsche Sporthochschule,C.Diemweg 2,D-5000 Köln, W. Germany, and M. Höckel, Universitätsfrauenklinik Mainz.

In 100 μl of human serum long chain non-esterified fatty acids are determined by high performance liquid chromatography with fluorescence detection. Using pre-chromatographic techniques which are outlined in the abstract by M. Höckel, W. Dünges et al., the biogenic saturated and non-saturated C_{12}- to C_{24}-acids are determined after reaction with 4-bromomethyl-7-methoxycoumarin (Br-Mmc) in place of methyl iodide. The reaction is carried out with 7-μl-solutions after addition of potassium carbonate and a crown ether in a microefluxer. The Mmc-esters are separated with a column containing C_{18}- brushes using a methanol/water gradient. A critical comparison of gas chromatography, high performance liquid chromatography and thin layer chromatography is presented. This seems to be of interest as identical conditions of sample preparation were applied.

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HPLC ANALYSIS OF MINOR COMPONENTS IN OILS. Fedeli Enzo and Cortesi Nicola, Stazione Sperimentale Oli E Grassi, Via G. Colombo, 79-20133 Milano, Italy.

The present paper is an attempt to obtain a full characterization of vegetable oils using HPLC. Minor components of the oils are the subjects of the HPLC determinations, partly done on the oil itself, partly on their unsaponifiables. Direct analysis by HPLC can be done on the oil: tocopherols and other phenolic components are analyzed and quantitatively determined. In particular, phenols and phenolic acids present in olive oil have been determined. Sterols can be determined on the unsaponifiable; Δ^7- and Δ^5-sterols are separated and determined. Again, operating on the unsaponifiables, the different tocopherols are separated and quantitatively determined.

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ARGENTATION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF METHYL ESTERS. C.R. Scholfield, Northern Regional Research Center, AR,SEA,USDA, 1815 North University, Peoria, IL 61604.

Argentation chromatography with silver ion on a macroreticular exchange resin has been applied to high-performance liquid chromatography to achieve more efficient and more rapid separations than were possible on previous low-pressure columns. Methyl oleate is eluted in 3.5 min and methyl linoleate in 30 min. Methyl linolenate, which at room temperature is bound too strongly to the resin for practical chromatographic separations, is recovered at higher temperatures. Procedures are described for elution of metyl linolenate and more rapid separation of dienes by programming column temperature from 25 to 70 C. Samples from ca. 0.025 to 8 μl can be analyzed on a 2 mm ID x 61 cm column. Two 7 mm ID x 61 cm columns in series have been used to separate 100-μl samples into fractions for further analysis. Although refractometric area percent corresponds fairly well to weight percent, the agreement is considerably improved by correcting for difference in refractive index of the components.

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SEPARATION OF GLYCEROL- AND CHOLESTEROL ESTERS BY HIGH EFFICIENT LIQUID AND GAS CHROMATOGRAPHY. Jiří Coupek, Laboratory Instruments Works, 16203 Prague 6, Na okraji 335, Czechoslovakia, and Premysl Mares, Medical Faculty of Charles University Prague, Czechoslovakia.

A method for quantitative gas chromatography analysis of plasma lipids, especially of free cholesterol, cholesterol and glycerol esters, permits a determination of lipid classes as well as their fractions without previous derivatization. The mutual effect of individual components of neutral lipid spectrum on the recovery was examined. Results obtained served as a basis for a comparison of the gas chromatography method with HPLC technique using new Czechoslovak reversed phase sorbents Separon with extremely high separation efficiencies. Isolation of lipid classes via adsorption and reversed phase liquid chromatography and the separation of individual components in the naturally occuring complex mixtures are being discussed in detail and demonstrated on examples. The HPLC resolution of a model system of cholesterol esters of fatty acids C_{14}-C_{20} was followed using chemically-bonded stationary phases with increasing carbon content while the silanol groups were substituted by methylation. The effects of polarity of covalently bonded phases has been investigated and compared with the adsorption chromatography on non-modified spherical microparticular silica Separon SI VSK. In order to speed up the analysis, the flowrate effects in the packed columns 100–250 mm long were studied.

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RAPID ANALYSIS OF SERUM LIPOPROTEINS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. I. Hara, M. Okazaki and Y. Ohno, Tokyo Medical and Dental University, Kohnodai, Ichikawashi, Chiba Prefecture, Japan 272, and K. Sakane, Tokyo Soda Mfg. Co.

The analysis of human serum lipoproteins by High Performance Liquid Chromatography (HPLC) has been investigated little. In the present research, rapid analysis of human serum lipoprotein was achieved using the new column system for aqueous solution which has columns of different pore sizes connected in series. The human serum lipoproteins, VLDL, LDL and HDL, were prepared by ultracentrifugation according to Havel's method and also tested by the immunological methods. Each lipoprotein dissolved in 0.15 M NaCl was applied to HPLC and eluted by 0.15 M NaCl with the flow rate of 1.1 ml/min under 70-80 Kg/cm^2. The lipoprotein fractions were monitored by the absorbance at 287 nm. Each lipoprotein fraction showed distinctly different elution time, and the mixture of these three lipoprotein fractions gave three separated peaks. The procedure for the analysis of lipoprotein was performed in less than one hour. HPLC may become the most rapid andconvenient method to assay the lipoprotein components and their homogenieties.

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THE STAIN REMOVAL INDEX (SRI): A NEW REFLECTOMETER METHOD FOR MEASURING AND REPORTING STAIN REMOVAL EFFECTIVENESS. O.W. Neiditch, K.L. Mills and G. Gladstone, Lever Brothers Company, 45 River Road, Edgewater, NJ 07020.

The development of laundry stain removal test methods is receiving increasing attention in task groups of three major standards developing organizations in the U.S. The need for such test methods is reflected in the proliferation of products for presoaking or pretreatment of stained laundry items prior to washing or for addition to the main wash solution to help insure complete removal of stains. Proposed test methods involve: (1) a standardized staining procedure, (2) pretreatment or presoaking of the stains, and/or (3) a wash in a detergent solution which may include an additive, (4) visual or instrumental evaluation of the degree of stain removal, and (5) calculation of percent stain removal when instrumental evaluation is used. Calculation of percent stain removal involves the use of the reflectance value of the applied stain. This can lead to anomalies in results from to the failure of reflectance readings to be directly proportional to the amount of stain applied. The "Stain Removal Index" defines the relationship of the appearance of the treated and washed stained fabric to the appearance of an unstained fabric of the same material.

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A MOTOR OIL SOIL FOR DETERGENCY TESTING. Lee Matheson and Garland Smith, Conoco, Inc., Chemicals Res., P.O. Box 1247, Ponca City, OK 74601.

A soil for detergency testing has been devised which is a blend of motor oil base stocks. The soil consists of a 1:1 mixture of Bright Stock and Pale Oil and contains none of the myriads of additives which cause actual motor oils to be quite nonstandard in composition. Therefore, it allows one to simulate motor oil response in detergency without the ambiguities produced by additives. Further,

Bright Stock is a deep amber colored material and requires no dyeing or staining which always adds some uncertainty in detergent testing. Tergotometer results show a definite response to surfactant types using this soil and the results are compared and contrasted with other soils. A real advantage of this soil is a remarkably good reproducitility of soiling and cleaning.

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A PRACTICAL METHOD FOR THE EVALUATION OF FOAMING PERFORMANCE OF SOAP IN HARD WATER. Joan W. Koppenbrink, Armour Research Center, 15101 N. Scottsdale Rd., Scottsdale, AZ 85260.

A method for evaluating the foaming or lathering performance of soaps in hard water has been developed. This technique involves measuirng the volume of foam generated when a stoppered graduated cylinder containing a soap solution in hard water is inverted a standard number of times. Foam volume is measured 30 seconds after the mixing is completed and 4.5 minutes later. A range of soap concentrations is used to locate foam volume profiles for the soap systems of interest. Mathematical treatment of the data provides numerical values which describe the maximum foam volume attainable in the system, the minimum soap concentration that will yield foam, the concentration of soap required to generate stable foam, and the concentration of soap which is sufficient to produce a given volume of foam. This method has proven to be reproducible, sensitive to differences between soap systems and easily performed with standard laboratory equipment. Foaming qualities of soaps made from a variety of tallow/cocoa ratios and several commercially available soap products have been evaluated with this technique.

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EFFECT OF SURFACTANTS AND ENZYMES ON DETERGENCY. Osamu Okumura, Hiroshi Nishio, Isao Amano and Kazuaki Fukano, Lion Fat & Oil Co. Ltd., 13-12, 7-chome, Hirai, Edogawa-Ku, Tokyo, Japan.

Phosphate regulation has been enacted in Japan requiring P_2O_5 levels at 15% in detergents by 1975, P_2O_5 at 12% by 1976 and P_2O_5 at 10% by this year. We studied the effect of proteolytic enzymes on detergency as phosphate substitutes or detergent auxiliary in Japanese washing conditions where the washing liquors are used without special heating, i.e. at 5–35 C. First, detergency was evaluated by artificially soiled cotton swatches using washing liquor containing LAS-Na 200 ppm, STP 226 ppm, sodium silicate and sodium carbonate 107 ppm, plus 6 kinds of proteolytic enzyme 0-2.0 x 10^{-2} Anson unit/1. The best result was obtained in Alcalase M among the six enzymes, and detergency was almost saturated at enzyme 6.7 x 10^{-3} AU/1 dosage level in every case, suggesting that there are some interactions between detergent components and enzyme, and specificity to substrate of enzymes. Next, anionic surfactants such as LAS-Na, AOS-Na and AES-Na, phosphates such as tetra-sodium pyrophosphate (TSPP) and penta-sodium tripolyphosphate (STP) and Alcalase M were combined with each other. Detergency, stability of enzyme in washing liquor and foam height during washing were studied as well as the effects of pre-soaking time, washing temperature and water hardness. The higher the stability of enzyme, the higher the detergency obtained and the AOS-Na-TSPP-Alcalase M system showed the best result. Also, this system was found to have the highest foam height, but LAS-Na-STP the poorest. This suggests there is some formation of a complex between anionic surfactant and enzyme, and the solubility, stability and surface activity of this complex as well as the binding power and capacity of phosphate for heavy metals affects detergency and foam ability.

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THE DETERMINATION OF OPTICAL BRIGHTENERS IN LAUNDRY DETERGENTS BY REVERSE PHASE AND ION PAIR HPLC. Bruce Paul McPherson and Nicholas Omelczenko, Colgate-Palmolive Company, 909 River Road, Piscataway, NJ 08854.

Seven commonly used optical brighteners can be qualitatively or quantitatively determined using C_{18}, C_8 or C_2 reverse phase HPLC columns. Mobile phases consist of acetonitrile and methanol in water with phosphate buffer; quaternary ammonium salts are added for ion pair formation if desired. UV detection at 340 nm gives more than adequate sensitivity for all current formulations. Sample preparation is simple and all components can be eluted within 15 min. Powdered and liquid detergents, fabric softeners, and bleach boosters have been routinely analyzed and results closely agree with those obtained using thin layer chromatography and densitometry.

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ETHYLENE OXIDE OLIGOMER DISTRIBUTIONS IN NONIONIC SURFACTANTS VIA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). M.C. Allen and D.E. Linder, Conoco, Inc., P.O. Box 1267, Ponca City, OK 74601.

An HPLC method using adsorption columns combined with linear gradient elution has been developed for the determination of ethylene oxide (EO) distribution in nonionic surfactants. The quantitative ethoxylate adduct distribution in single-carbon number and mixed-carbon number primary alcohol-based samples can be obtained. The HPLC method is also applicable for determining the molar EO distributions in diverse ethylene oxide adduct compounds such as alkylphenol ethoxylates, branched alcohol ethoxylates and secondary alcohol ethoxylates. Nonionic surfactant samples containing adducts up to 25 m have been successfully separated and the individual adducts quantitated.

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HPLC ANALYSIS OF INTACT AND PARTIALLY BIODEGRADED LINEAR ALKYLBENZENE SULFONATES. D.E. Linder and M.C. Allen, Conoco, Inc., P.O. Box 1267, Ponca City, OK 74601.

A high performance liquid chromatography (HPLC) method for the determination of intact and partially degraded linear alkylbenzene sulfonate (LAS) was developed. The LAS degradation products resulting from a Semicontinuous Activated Sludge (SCAS) and a DieAway CO_2 study were quantitatively determined by HPLC equipped with a reversed-phase column using a tetrahydrofuran/water/Pic A solution as the mobile phase. The column effluent was monitored with a variable wavelength ultraviolet detector operating at 224 nanometers in series with a fluorescence spectrophotometer. The fluorescence spectrophotometer was operated at an excitation wavelength of 232 nanometers and an emission wavelength of 290. Compounds investigated included both single- and mixed-carbon range materials. The HPLC method shows good correlations with MBAS for intact LAS material. The most significant advantage of this method over MBAS is its ability to quantitatively determine partially degraded intermediates and the disappearance of those intermediates. This method, when using the fluorescence detection system, is selective for sulfonated ring structures with more than one alkyl carbon. The sensitivity of the method is in the 0.2 ppm range. It can be used not only for samples from laboratory experiments but also for sewage plant influents, effluents and river waters.

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COMPOSITION OF SOYBEAN LECITHIN. C.R. Scholfield, Northern Regional Research Center, AR, SEA, USDA, 1815 North University, Peoria, IL 61604.

Commercial lecithin is a complex mixture containing ca 65-75% phospholipids together with triglycerides and smaller amounts of other substances. The lecithin marketed today is predominantly from soybeans. Although its general composition has been known for many years, the nature and amount of many smaller components are still uncertain. The major phospholipids include phosphatidyl choline, phosphatidylethanolamine and inositol-containing phosphatides. Other substances reported include carbohydrates, pigments, sterols and sterol glycosides. More recently, the presence of other phospholipids and glycolipids has been detected by thin-layer and liquid column chromatography. This paper will review the nature of the compounds found in soybean lecithin and our present knowledge of its composition.

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DEGUMMING OF SOYBEAN OIL: EFFECT OF OPERATIONAL PARAMETERS ON LECITHIN REMOVAL AND QUALITY. G.R. List, J.M. Avellaneda and T.L. Mounts, Northern Regional Research Center, AR, SEA, USDA 1815 North University, Peoria, IL 61604.

A commercially extracted crude soybean oil (570 ppm phosphorus, 1.74% acetone insolubles) was degummed in the laboratory under a wide range of reaction parameters (water concentration, temperature, time, and agitation). The reaction parameters were correlated with phosphorus removal from the oil as well as with color and acetone-insoluble content of the gum fraction. Efficiency of removal of phosphorus-containing compounds was independent of time, temperature and agitation. Water concentration had the most significant effect on removal of phosphorus from crude soybean oil. Some darkening of the lecithin was observed at temperatures above 60 C and with increased agitation. Individual parameters of time and temperature had relatively little effect on the acetone-insoluble content of the gums. Low agitation rates and water in concentrations of other than 2% (either more or less) entrained excessive amounts of oil in the gums. Under our experimental conditions, the optimum parameters with respect to phosphorus removal, lecithin color, and acetone-insoluble content are estimated to be: time–short (15 min); agitation–moderate to rapid (400 rpm); temperature–60 C; water concentration– 2% or an amount close to the phosphatide content of the crude oil. Bleaching with hydrogen peroxide to produce single-bleached lecithin was investigated. From limited data it appears that when degumming and bleaching are performed simultaneously, effectiveness of bleaching is a function of peroxide concentration and time. Thus, longer degumming times are required to prepare bleached lecithin compared to unbleached products.

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CORN LECITHIN. Evelyn J. Weber, USDA-SEA-AR-NCR, S320 Turner Hall, University of Illinois, Urbana, IL 61801.

In recent years, soybeans have been the sole source of commercial lecithin in the U.S. With the phenomenal growth now occurring in the demand for corn sweeteners, other products of the corn refining industry, such as corn lecithin, may become more available and competitive. The physical properties of a commercial lecithin are determined by the proportions of the various phospholipids and other lipids that it contains. We have analyzed a commercial corn lecithin. The major phospholipids were phosphatidylcholine (39%) and phosphatidylinositol (21%). Some degradation during processing was indicated by the higher percentages of phosphatidic acid and lyso lipids found in the commercial lecithin compared to phospholipids isolated from corn inbreds. The fatty acid compositions of corn oil (triglycerides) and the phospholipids are controlled by the genetic background of the corn, but each lipid class has its own distinctive fatty acid pattern. Fatty acid placement in all the corn phospholipids follows the usual distirbution for plant lipids of more unsaturated fatty acids in the 2-position than in the 1-position.

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INSTANTIZATION OF FOOD WITH LECITHIN IN MIXING PROCESS. K. Strauss, c/o Lucas Meyer, Ausschläger Elbdeich 62, D-2000 Hamburg 28, Germany.

Instantization comprises the process steps of agglomeration and surface coating by lecithination. Based on theoretical considerations, agglomeration has so far been regarded as an essential process step. It could have been shown, however, that lecithination as the only process step that will impart instant properties that are nearly as good as those obtained by agglomeration plus lecithination, provided that the proper treatment is applied to the product. Instantization of various food products by lecithination with a lecithin powder (Metarin) was achieved in a double jacketed agitated drum dryer. The lecithin was admixed at preferably 60 C. Examples for instantized products are soyprotein isolate, calcium caseinate, coconut powder, potato starch, dried soup mixes, and powdered coffee extract. The instant properties were characterized by the wetting time, which could be reduced to below 30 seconds in all cases. The instant properties remained stable on storage for at least 3 months. The main advantages of this method of instantization are cost and energy savings.

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SUNFLOWER LECITHIN. W.H. Morrison III, USDA, SEA, R.B. Russell Agricultural Research Center, Field Crops Laboratory, P.O. Box 5677, Athens, GA 30604.

Due to the increased domestic production of sunflowerseed oil, by-products of oil processing such as meal and lecithin will be in a greater supply. This presentation will cover some of the properties and processing details of lecithin production. Lecithin content varies according to the type of sunflowerseed produced. Removal of this material is greatly affected by seed condition and processing conditions. Conditions which favor optimum removal of phospholipids are also those for optimum oil quality.

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LECITHIN EXTRACTION, CHARACTERIZATION, AND USE WITH EMPHASIS ON GLANDLESS COTTONSEED AS A COMMERCIAL SOURCE. J.P. Cherry and M.S. Gray, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179; and L.A. Jones, NCPA, Memphis, TN 38112.

Industrial lecithin (crude polar lipids) can be fractionated as phospholipids and glycolipids after removal of neutral lipids and protein-containing contaminants. The polar lipids are very reactive, causing difficulty in extracting and purifying them from oilseeds. Their purity, and special properties, can be improved by a number of methods including solvent fractionation, hydrogenation, sulfonation, and ethoxylation. Studies are determining the role of the polar lipids of lecithin in: (a) synthesis of triglycerides in maturing seeds, (b) structure of biological membranes, and (c) molecular basis of functionality of food ingredients. High surface activity of both polar and non-polar groups makes them reactive with both oil and protein and thus they serve as excellent emulsifying agents in food systems; they also slow auto-oxidation and enzyme hydrolysis of fats. Cottonseed lecithin is low in highly unsaturated linolenic acid and has been shown to prevent flavor deterioration of soybean oil; it has also been used to stabilize high-boiling sunflower oil to color change. Gossypol binds to lecithin economically, negating glanded cottonseeds as a commercial source of this product. New cultivars producing glandless, or gossypol-free, cottonseeds have potential as commercial sources of edible-grade lecithin.

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COMPARISON OF METHODS FOR THE ANALYSIS OF PHOSPHORUS AND LECITHIN IN RAPESEED OIL. James K. Daun and Lynda D. Davidson, Canadian Grain Commission, Grain Research Laboratory, Room 1308, 303 Main Street, Winnipeg, Manitoba, R3C 3G9. Canada; John A. Blake and Wo Yeun, POS Pilot Plant Corporation, Canada Packers Limited.

In a collaborative study, methods for determining phosphorus and lecithin in crude and refined rapeseed oil were compared. The accuracy and precision, speed, and usefulness of the AOCS official procedure is compared with three different combustion/digestion procedures followed by spectrophotometry, as well as with instrumental procedures using molecular emission cavity analysis and atomic absorption spectrophotometry. The relationship between phosphorus and lecithin (acetone insolubles) in rapeseed oils is also examined. A rapid procedure for determining acetone insolubles using quantitative thin-layer chromatography is described.

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OBTAINING OF PHOSPHATIDYLCHOLINE. A. Nasner, c/o Lucas Meyer, Ausschläger Elbdeich 62,D-2000 Hamburg 28, Germany.

The specification phosphatidylcholine does not characterize a homogenous substance but, due to the variability of the fatty acid residue, a substance group. Phosphatidylcholines are wax-like hygroscopic substances without defined melting points. In changing composition, they are components of the phospholipids that are found in nature and are existing as accompanying substances of fat in all vegetable and animal cells, including monocellular organisms. Therefore it is possible to obtain phosphatidylcholine by fractionation; in doing so no pure substances will be obtained by solvent separation due to the amphiphile properties of the phospholipids. Purification, however, is possible by adsorbate fractionation. Several possibilities are described for the synthetic preparation. For the partial synthesis, mainly encymatic methods in different variations are applied. Original products are, for example, fatty acid triglycerides, which can also occur as intermediates of the total synthesis or other phospholipids. Because of their properties, phosphatidylcholines are not only used in emulsifying mixtures, but also as active components that are donors for choline and essential fatty acids.

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MEASURES OF OXIDATIONS IN FATS. Fedeli Enzo and Gasparoli Ada, Stazione Sperimentale Oli e Grassi, Via G. Colombo, 79-20133, Milano, Italy.

The measure of the oxidation conditions of a fat is prohibitive because of the numerous parameters involved in the autoxidation mechanisms. Most of the parameters are bound to the "history" of the fat, even when the fat has been refined. We have studied a dynamic measure in contrast to the static ones to predict the stability. The methods involved are based on HPLC determinations and on spectroscopic observation of the samples. The HPLC method in particular consents not only a measure of the peroxides present at a given time but takes into account decomposition products formed. The values taken from HPLC and spectroscopy observations are used as kinetic parameters to foresee the stability of a fat.

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THE MECHANISM OF THE REARRANGEMENT OF PENTADIENYL HYDROPEROXIDES. H.W.S.-Chan, J.A. Matthew, and D.T. Coxon, ARC Food Research Institute, Colney Lane, Norwich, Norfolk, NR4 7UA, United Kingdom.

Pentadienyl hydroperoxides formed by the oxidation of methyl linoleate (methyl hydroperoxylinoleates) underwent a rearrangement reaction in which a single isomer gave rise to a mixture of four hydroperoxide isomers. Solvent dependence, inhibitory effects of anti-oxidants, and promotive effects of radical generators pointed to a radical-chain mechanism. The oxygen atoms of the –OOH group exchanged with atmospheric molecular oxygen during rearrangement of the hydroperoxides. Evidence is presented that the reaction was an example of a rearrangement in which molecular oxygen was the migrating group. The implication of the rearrangement reaction in other autoxidative processes will be discussed.

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EPR FLOW AND SPIN-TRAPPING TECHNIQUES APPLIED TO PROBLEMS IN LIPID OXIDATION. K.M. Schaich and D.C. Borg, Medical Department, Brookhaven National Laboratory, Upton, NY 11973.

Free radical production in the initiation and propagation phases of lipid oxidation has been studied by two special electron paramagnetic resonance (EPR) techniques: slow flow at Q-band (35 GHz) and spin-trapping in conjunction with flow at Q-band and in static systems at X-band (9.5 GHz). Radicals in model systems containing methyl linoleate or linoleic acid were generated chemically (titanium [III] or iron [II or III]: hydrogen peroxide or linoleate hydroperoxide), electrolytically, or by "spontaneous" autoxidation. Distinct differences were demonstrated in the abilities of the spin traps DMPO (dimethyl pyrrolidine-N-oxide), PBN (phenyl-t-butyl nitrone), and t-NB (nitrosobutane) to trap initiating species e^- and •OH versus the primordial lipid alkyl-radicals or

secondary lipid oxy-radicals. Whereas DMPO preferentially trapped electrons or oxy-radicals, nitrosobutane trapped secondary alkyl-radicals from the solvent, metal chelator, or other components added to the system. PBN proved to be the most versatile agent, trapping initiating species, lipid alkyl- and oxy-radicals, and other secondary radicals under a variety of reaction conditions. For all three spin traps, the adduct(s) observed depended on the reaction stage monitored, i.e., the rate of flow or time of addition to spin trap to a static reaction mixture, as well as on the nature and relative proportions of reactants in a given system. Q-band flow studies utilizing a range of flow rates (<0.5 to ~3.0 ml/min) with and without spin traps provided enhanced resolution of overlapping spectra as well as important supplementary information about radical stabilities, competitions, and reaction sequences. It was concluded that while an appropriate spin trap may be applicable for studying specific radicals in a single defined reaction, the most accurate and complete understanding of the radical process comprising a complex reaction sequence such as that expected in lipid oxidation requires coordination of data obtained from several spin traps with data obtained from flow experiments. This work was supported by the U.S. Department of Energy.

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ESTIMATION OF THE PROCEEDING OF TISSUE LIPID PEROXIDATIONS BY THE MEASUREMENT OF CHEMILUMINESCENCE. Teruo Miyazawa and Takashi Kaneda, Department of Food Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumi-dori Amamiya-machi, Sendai, Japan 980.

The objective is to evaluate the possibility for measuring the progress of tissue lipid peroxidations and aging by the detection of tissue spontaneous chemiluminescence with a single photon counting system. The results were summarized as follows. (a) Chemiluminescence intensities of rat tissue (blood, liver, lung, heart and kidney, etc) homogenates were increased characteristically by the feeding of autoxidized linseed oil (POV 400, 1/5 vol of LD_{50} per day) or the irradiation (90 hours) with ultraviolet light (15 W). The chemiluminescence increased was found to be closely related to the tissue peroxidation proceedings characterized by TBA value and POV. (b) On administrating CCl_4 (0.5 ml/100 g body wt) of rats, the emission intensity of CCl_4-induced fatty liver homogenate was obviously enhanced in parallel with the growth of TBA value. The proceeding of lipid peroxidation in CCl_4-induced fatty liver was confirmed by the counting of emitted chemiluminescence. (c) Aging degrees of tissues could be discriminated between two-month- and eleven-month-old rats by means of counting the spontaneous emitted-light intensities of the homogenates. This discrimination could not be achieved sufficiently by TBA assay. High sensitivity of this detection system for the estimation of tissue aging degrees is suggested.

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MODEL EXPERIMENTS ABOUT THE FORMATION OF VOLATILE CARBONYL COMPOUNDS. W. Grosch, P. Schieberle, and G. Laskawy, Lichtonbergstrasse 4, D-8046 Garching, West Germany.

The aerobic breakdown of both 9-hydroperoxyoctadeca-*trans*-10,*cis*-12-dienoic acid (9-LOOH) and 13-hydroperoxyoctadeca-*cis*-9,*trans*-11-dienoic acid (13-LOOH) in the presence of copper ions was studied at moderate temperatures. In both cases hexanal resulted as the main product of the volatile carbonyl compounds. In the experiment with 9-LOOH, only traces of 2,4-decadienal, which is theoretically derived from the 9-LOOH, were detected. Antioxidants (BHA, BHT, α-, δ- and θ-tocopherols) increased the concentration of 2,4-decadienal and lowered that of hexanal during the decomposition of 9-LOOH. The breakdown of 13-LOOH to hexanal was not altered by the antioxidants. Analysis of the autoxidation products of 2,4-decadienal showed hexanal and peroxides of unknown structure as the main components. The results are compared with other data and a pathway for the formation of hexanal and 2,4-decadienal is discussed.

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FURTHER STUDIES OF A MODEL FOR LIPID HYDROPEROXIDE DEGRADATION: CHARACTERIZATION OF NEW PRODUCTS AND THE NON-PARTICIPATION OF SUPEROXIDE ANION IN THE FORMATION OF SECONDARY OXIDATION PRODUCTS. H.W. Gardner and P.A. Jursinic, Northern Regional Research Center, 1815 N. University Street, Peoria,, IL 61604.

Previously we reported that 13-L(S)-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid decomposed in the presence of O_2 and a $FeCl_3$-cysteine catalyst into optically active oxoepoxyene and hydroxyepoxyene fatty acids. Since the epoxy groups were located almost exclusively at carbon-12 and -13, it is probable that a 12,13-epoxyallylic radical originated from rearrangement of an alkoxy radical generated from the hydroperoxy group. On the basis of products obtained, it was proposed that the epoxyallylic radical added a molecule of O_2 at either carbon-9 or -11, resulting in a secondary hydroperoxide at these carbons. The secondary hydroperoxides were eventually decomposed by the catalyst to oxo-epoxyene and hydroxyepoxyene fatty acids. Previously we were unable to detect two of the eight *trans*-epoxides predicted by theory. Now, we have identified both 9-hydroxy-*trans*-12,13-epoxy-*trans*-10-octadecenoic acid and 11-oxo-*trans*-12,13-epoxyoctadec-9-enoic acid, the latter being a novel compound. The formation of hydroperoxyepoxyene fatty acid intermediates was not due to oxidation by superoxide anion, because superoxide was not observed with the acidic conditions (pH ca. 3–6) used to obtain the epoxides. At alkaline pH's, superoxide anion was readily generated, but products shifted to the almost exclusive formation of hydroxy-octadecadienoic acid. Superoxide formation was found to be correlated with the pK_2 of cysteine; thus, the reductant probably was thiolate anion.

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QUANTITATIVE ANALYSES OF HYDROPEROXIDES BY HIGH PRESSURE LIQUID CHROMATOGRAPHY OF AUTOXIDIZED AND PHOTOSENSITIZED-OXIDIZED FATTY ESTERS. W.E. Neff and E.N. Frankel,* Northern Regional Research Center,AR,SEA,USDA, 1815 North University, Peoria, IL 61604.

A high-pressure liquid chromatography (HPLC) method is described for the determination of the isomeric hydroperoxide composition of oxidized fatty esters. The fatty esters are hydrogenated and the mixtures of hydroxystearates are concentrated by partial removal of unoxidized esters and complete removal of polar materials. Isomeric hydroxystearates are then separated on a porous microparticulate adsorption (10 μ) column. Elution with 0.25% isopropyl alcohol in n-hexane is monitored at 212 nm. The accuracy of the method was checked with known mixtures of synthetic hydroxystearates. The isomeric hydroperoxide compositions of oxidized methyl oleate, linoleate, and linolenate determined by HPLC were in good agreement with previous analyses by gas chromatography-mass spectrometry.

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ANALYSIS OF OLEATE, LINOLEATE, AND LINOLENATE HYDROPEROXIDES IN OXIDIZED ESTER MIXTURES. Seyed H. Fatemi and Earl G. Hammond,* Room 200, Dairy Industry Building, Iowa State University, Ames, IA 50011.

The hydroperoxides in oxidized mixtures of methyl oleate, linoleate, and linolineate were analyzed by reducing the hydroperoxides to the corresponding hydroxyesters and separating the hydroxyesters from the unoxidized esters by thin layer chromatography. The hydroxyester from linolenate was separated from the other hydroxyesters by thin layer chromatography on silver ion plates. The hydroxyesters were converted to TMS-derivatives. The TMS-oleate and TMS-linoleate were separated by gas chromatography, and all the TMS-derivatives were quantified by gas chromatography. The relative rates of oxidation of methyl oleate, linoleate, and linolenate in binary and trinary mixtures were about 1:10.3:21.6 at both 21 and 28 C, and these ratios did not change with the extent of oxidation up to peroxide values of at least 70 meq/kg. The amounts of the peroxides formed in the oxidation of soybean and olive oils were similar before and after randomization and similar to corresponding methyl ester mixtures.

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THE APPLICATION OF DIELECTRIC PERMEATIVITY MEASUREMENTS TO INVESTIGATION OF THE INITIATION STAGE OF AUTOXIDATION OF OLEYL ALCOHOL. Józef Sliwiok and Teresa Kowalska, Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland.

The results show the possibility of application of the dielectric permeativity measurements to description of the initiation stage of autoxidation with oleyl alcohol, taking into consideration intermolecular interactions through H-bonds. In our work, samples of bicomponent systems containing oleyl alcohol and higher fatty alcohol were applied (the higher fatty alcohols with an even number of carbon atoms in a molecule between C_{12} and C_{18}). The consecutive application of higher fatty alcohols as one component of our system aimed smoothly to modify the concentration of H-bonds able to interact in the reaction atmosphere. The complex use of the peroxide number value, and the dielectric permeativity measurements with the unoxidized samples and with those after one day of oxidation at 50 and 60 C introduced certain methodical novelty. The results allowed the formulation of the following hypothesis. At the initiation stage of autoxidation with monounsaturated substances, the influence of interactions through H-bonds demonstrates an inhibiting effect, contrary to what was observed at the propagation stage of autoxidation.

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RETROSPECTIVE OVERVIEW/A LOOK BACKWARD AT THE 1970s. E.C. Leonard, Humko Sheffield Chemical, P.O. Box 398, Memphis, TN 38101.

A statistical summary of the fatty acid business during the 1970s will be presented, along with a discussion of the price pattern during that decade of the principal raw material feedstocks for the fatty acids and fatty acid derivatives. A comparison will be made of the roster of producers in 1970 and 1980—additions, deletions, and ownership changes.

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TALL OIL FATTY ACIDS—1990. Maurice J. Kelly. Marketing Division, 12th Floor, Hercules Incorporated, 910 Market Street, Wilmington, DE 19899.

The production of tall oil fatty acids is tied to a feed material that is not expected to grow more than 3 to 4% over the next several years; however, there appears to be a trend for mills to increase their crude tall oil recovery to improve the economics of their operation and to meet ecology requirements. This has offset such factors as the increased use of hardwood, of lumber chips instead of logs, and increased recycling of waste paper. The increased use of tall oil fatty acids in the chemical intermediate market will continue. The paper shows the growth of such chemical uses as dimer acids for polyamide resins; C-21 diacid surfactants; and oleic, linoleic, and conjugated linoleic acids by 1990.

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CURRENT AND FUTURE MARKETS FOR FATTY ACIDS IN THE RUBBER INDUSTRY. L.G. Parkinson, Hull and Company, 5 Oak Street, Greenwich, CT 06830.

Fatty acids from animal and vegetable sources are used both as acids and fatty acid derivatives in elastomeric compositions, largely as part of the vulcanization system, and as soaps in the emulsion polymerization method of manufacturing synthetic rubbers. Additional small amounts are consumed in plasticizers and specialty additives to modify polymeric systems. The size of these markets and growth prospects for the United States usage are discussed and data representing estimated consumptions by end-use of fatty acid type or derivative type are shown. Economic factors influencing growth patterns are reviewed.

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METALLIC STEARATES—TODAY THRU 1990. Thomas J. Gibbons, Diamond Shamrock Corporation, 350 Mt. Kemble Avenue, Morristown, NJ 07960.

This paper reviews the current and projected demand for metallic stearates in various end-use segments. Each end-use market will be briefly reviewed as to the type of metallic stearates used and what the projected growth rates will be through 1990. The process for manufacturing precipitated, dispersed, and fused type stearates will be highlighted as well as the current capacity situation for these products. Selected end-use formulations will also be provided. A profile of the major soap producers will be shown as well as some of the problems they face from competitive products and technologies.

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FATTY CHEMICALS FOR SYNTHETIC LUBRICANTS IN THE 1980s. S.E. Gloyer and T.E. Breuer, Humko Sheffield Chemical, P.O. Box 398, Memphis, TN 38101.

The area of synthetic lubricants has been recognized for a number of years as corresponding with a potentially significant demand for fatty acids. The paper examines the growth of the synthetic lubricant field and the growth of those parts of it that utilize fatty acids. During the past several years, questions have been raised concerning the ability of the fatty acid industry to provide the volumes of fatty materials projected to be needed by the synthetic lubricant market. Present and potential sources of the fatty acids used in the synthetic lubricant area are evaluated. Views on fatty acid demands by the synthetic lubricant producers and trends in synthetic lubricants in the 1980s are presented.

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THE HUMAN FOOD MARKET FOR FATTY ACIDS. Robert Stutz and J.M. Hesser, Hesser & Associates, Inc., 10100 Santa Fe Drive, Overland Park, KS; Maurice J. Kelly and William T. McNabola, Hercules, Inc.

The market demand for fatty acids and their derivatives in human foods is projected for the 1980s, with particular emphasis on new applications. A breif review of this market during the 1970s is also presented.

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ECONOMIC FORECASTING FOR THE FATTY ACID INDUSTRY. E.C. Leonard, Humko Sheffield Chemical, P.O. Box 398, Memphis, TN 38101.

A method for forecasting U.S. fatty acid demand (excluding tall oil fatty acids), based on a correlation with a composite econometric index, will be presented. Turning points in economic trends are among the most difficult aspects of economic forecasting. The paper will include a description of methodology which approaches the "turning point prediction" problem for the fatty acid industry.

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HYDROGENATION, PRACTICES UPDATE. Calvin T. Zehnder, Chemetron Process Equipment, P.O. Box 35600, Louisville, KY 40232.

Hydrogenation of edible oils has been practiced for many years and is the one truly chemical process of oil refineries. Many papers have been presented dealing with the specifics of the chemical reactions as related to various oils and characteristics desired of the end products. Current methods and equipment for carrying out this high economic-impact process will be reviewed. Special emphasis will be given to design consideration that will permit control of product quality, operational flexibility, and energy economies.

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LABORATORY-SCALE CONTINUOUS HYDROGENATION: EFFECT OF PRESSURE. J.M. Snyder, T.L. Mounts, C.R. Scholfield, and H.J. Dutton, Northern Regional Research Center, AR, SEA, USDA, 1815 North University, Peoria, IL 61604.

A laboratory-scale high-pressure continuous hydrogenation reactor was used to partially hydrogenate soybean oil with copper catalysts. The open-pipe-type reactor consisted of 40 feet of 1/16 inch (ID) stainless steel tubing in a constant-temperature, hot-air bath. A suspension of catalyst in oil was passed, at 2.2 ml/min, concurrently with hydrogen gas, at 100 ml/min, through the reactor. Data were obtained to characterize effects on the kinetics and mechanism of the reaction by conducting experiments in a central composite design. The interaction of pressure (75 to 205 psig) with the other independent variables of temperature (155 to 225 C) and catalyst concentration (.15 to 1.85% wt/wt) was evaluated. Dependent variables studied were reaction rate, linolenate selectivity, *trans* formation, and formation of conjugated dienes. Studies outside the basic experimental design examined effects of pressure up to 500 psig, use of experimental as well as commercial copper catalysts, and comparisons with high-pressure batch reactions. The rate of reaction increases significantly with increases in pressure, temperature and catalyst concentration. Linolenate selectivity is high (9 to 10) and is not significantly affected by any of the process variables. *Trans*-isomer formation is not affected by process variables. Conjugated dienes are eliminated as products of the reaction when pressure is above 205 psig. Experimental copper-silica catalysts give a 1.6-fold increase in reaction rate over commercial copper catalysts.

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HYDROGENATION OF VEGETABLE OILS WITH SULFUR TREATED NICKEL CATALYSTS. R.R. Allen and J.E. Covey, Anderson Clayton Foods, W.L. Clayton Research Center, 3333 North Central Expressway, Richardson, TX 75080.

The hydrogenation of vegetable oils with nickel catalyst that has been treated with sulfur causes the rate of geometrical isomerization of the double bonds to increase. Also, the sulfur treatment of the catalyst decreases the activity for hydrogenation. These catalysts were studied by comparing the ratio of sulfur/nickel to the rates of hydrogenation and isomerization. Also, the positions of the geometrical isomers was determined. The results indicate two separate reactions were occurring: (1) isomerization due to the nickel/sulfur, and (2) hydrogenation-isomerization due to the nickel catalyst.

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A COMPARISON OF HYDROGENATION SELECTIVITY BETWEEN TRIGLYCERIDES AND FATTY ACID. R.C. Hastert, Harshaw Chemical Company, P.O. Box 22126, Beachwood, OH 44122.

Edible tallow and tallow fatty acids were hydrogenated at high temperature (216 C) and varying pressure (50 to 370 psig) to compare the *trans*-isomer and preferential selectivities of the two feedstocks. Differences in *trans*-isomer formation were evident between the feedstocks with the triglyceride generating more *trans*-isomers at all pressures. Increasing pressure suppressed *trans*-isomer formation on both feedstocks. Increasing pressure also decreased preferential selectivity on the triglyceride. Whereas no difference in preferential selectivity was evident among the fatty acid runs, even at the pressure extremes, this could have been due to analytical perception reflecting the small amount of polyunsaturates in the fatty acid feed. In order to more conclusively demonstrate triglyceride vs. fatty acid preferential selectivity, the experiment is being repeated, and will be reported, for soybean oil vs. soybean oil fatty acids.

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INFLUENCE OF REMAINING PHOSPHATIDES DURING HYDROGENATION OF SOYBEAN OIL. Ivar Ottesen and Bjørn H. Jensen, A/S Denofa Og Lilleborg Fabriker, Postboks 40, 1601 Fredrikstad, Norway.

We have noticed that the specifications of hardened soybean oil vary too much regarding melting point, iodine value, and solid fat content, even when the conditions during neutralization, bleaching, and hydrogenation are identical. Lecithin is a catalyst poison. By

adding different amounts of lecithin to an oil and measuring the catalyst consumption, it is possible to determine the poisoning factor. We have previously found this factor for stearyl-palmitoyl-lecithin to be 0.0008% Ni/ppm phosphorous. This means that the poisoning factor is the amount of catalyst that will be totally inactivated by one unit of catalyst poison. The poisoning factor of lecithin is small, but if adsorbed lecithin influences the selectivity of the catalyst, this could be an explanation for the variations. A deodorized soybean oil containing different amounts of added pure lecithin was hydrogenated. The trials were repeated with another soybean oil with and without an addition of soybean phosphatides. An addition of phosphatides to the oil prior to hardening resulted in a high amount of saturated acids, low *trans* content, a high melting point, and a change of the solid fat content. A poor triglyceride selectivity would explain our findings and the variations in the specifications, because of the variations in the phosphatide content in the oil prior to hardening. If the phosphatides adsorb to the outer part of the catalyst pore, this would have same effect as a narrowing of the pore, and result in a reduced triglyceride selectivity. A thorough neutralization and repeated treatments with lye will lower the amount of phosphatides in the oil before hydrogenation. The same effect can be achieved by increasing the amount of bleaching earth. By increasing the amount of catalyst, the selectivity will be better. As phosphatides seem to result in a reduced selectivity, a higher amount of catalyst can compensate for the effect of the phosphatides. When keeping the hardening time constant, 0.01% Ni compensates for 25 ppm phosphatides (~1 ppm phosphorous). This means that the compensation value is 12 times higher than the poisoning factor.

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HYDROGENATION OF METHYL SORBATE AND SOYBEAN ESTERS WITH POLYMER-BOUND METAL CATALYSTS. E.N. Frankel, J.P. Friedrich, and T.R. Bessler, Northern Regional Research Center, AR, SEA, USDA, 1815 North University, Peoria, IL 61604; N.L. Holy, Western Kentucky University.

New polymer-bound hydrogenation catalysts were made by complexing $PdCl_2$, $RhCl_3$ and $NiCl_2$ with anthranilic acid anchored to chloromethylated polystyrene. The Pd(II) and Ni(II) polymers were reduced to the corresponding Pd(O) and Ni(O) catalysts with $NaBH_4$. In the hydrogenation of methyl sorbate, these polymer catalysts were highly selective for the formation of methyl 2-hexenoate. The diene to monoene selectivity decreased in the order: Pd(II), Pd(O), Rh(II), Ni(II). The relative activity decreased in the order: Rh(II), Pd(II), Pd(O), Ni(II). In the hydrogenation of soybean esters, the Pd(II) polymer catalysts proved to be superior, because they were more active than the Ni(II) polymers and produced less *trans* unsaturation than the Rh(II) polymers. Hydrogenation with Pd(II) polymers at 50 to 100 C and 50 to 100 psi H_2 decreased the linolenate content to below 3%, and increased *trans* unsaturation to 15 to 20%. The linolenate to linoleate selectivity ranged from 2.1 to 2.8. Reaction parameters were analyzed statistically to optimize hydrogenation. On recycling through two or three hydrogenations of soybean esters, the 20 mesh Pd(II) polymer remained more active than the 100 mesh Pd(II) polymer. Hydrogenation with Pd(II) polymer catalysts probably involves in situ formation of Pd(O) polymer.

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DESATURATION OF ISOMERIC *TRANS*- AND *CIS*-OCTADECENOIC ACIDS BY RAT LIVER MICROSOMES, AND THE EFFECTS OF THESE ISOMERS UPON Δ5, Δ6 and Δ9 DESATURASES. M.M. Mahfouz and R.T. Holman,* The Hormel Institute, University of Minnesota, 801 16th Avenue N.E., Austin, MN 55912.

The rates and positions of desaturation of 12 labeled positional isomers of *trans*- 18:1 and of 8 isomers of *cis*-18:1 were studied, using enzymes of liver microsomes from EFA-deficient rats. Oleate substrate was used for optimizing conditions and as a model for comparison. Some isomers of t-18:1 gave mostly *cis,trans*-18:2; some gave mostly *cis,cis*-18:2; some gave mixtures of these; and some were not measurably desaturated. The site of desaturation was at position 9, indicating action by Δ9 desaturase. Rate of desaturation increased as the double bond was moved from position 9 toward either end of the chain. The pathway for conversion of *cis,trans*- to *cis,cis*-18:2 was examined. Some isomers of *cis*-18:1 were not desaturated; some were desaturated at low rates. Maximum rates occurred for Δ8 and Δ9 isomers by Δ5 and Δ6 desaturases, respectively. The *trans*-18:1 acids inhibited all three desaturases, and the *trans* double bond position influenced the degree of inhibition. The inhibition of Δ9 desaturase may be caused by the fact that *trans* acids are alternative substrates. The study shows that *trans*-18:1 acids can affect lipid metabolism through inhibition of desaturases, and suggests possible effects through the unusual 18:2 isomers produced.

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EFFECTS OF HYDROGENATED FATS CONTAINING *TRANS* FATTY ACIDS ON HEART MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION. Steven Royce and F.A. Kummerow, 205 Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, IL 61801.

This work is an extension of previous findings suggesting an adverse effect of dietary *trans* fatty acids on rat heart mitochondrial oxidative phosphorylation. Weanling, male Sprague-Dawley rats were fed two levels (5% and 15%) of three different sources of fat (olive oil, lard, and a formulated margarine base stock) for ninety days. All three sources of fat had similar levels of linoleic acid (approximately 10%). Olive oil (00) and lard (L) contained minimal amounts of *trans* fatty acids, whereas the formulated margarine base stock (FMS) contained approximately 40% *trans* fatty acids. Gas-liquid chromatography of fatty acids in heart lipid classes were determined along with several parameters of heart mitochondrial oxidative phosphorylation (i.e., oxygen uptake rates, ADP/O ratios, ATP synthesis rates, cytochrome c oxidase activity, and the ATP-ADP exchange reaction). The significance of the influence of dietary *trans* fatty acid on rat heart mitochondrial metabolism will be discussed.

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β-OXIDATION OF *TRANS*-OCTADECENOIC FATTY ACIDS BY HEART MITOCHONDRIA. Larry D. Lawson and Ralph T. Holman, The Hormel Institute, University of Minnesota, 801-16th Ave., N.E., Austin, MN 55912.

The coenzyme A esters of the Δ^4 through Δ^{15} positional isomers of *trans*-octadecenoic acid were prepared. The isomers were compared in their ability to be oxidized by rat heart mitochondria in the presence of L-malate, L-carnitine, and ADP. The oxygen uptake rates were measured polarographically using a Clark oxygen electrode. The oxidation rates were also compared to the coenzyme A esters of oleic, stearic, and palmitic acids. All oxidation rates were compared at several substrate/albumin ratios. The results showed a bimodal distribution of the oxygen uptake rates of the positional isomers, centering on the Δ^9 isomer, which was oxidized significantly slower than its nearest neighbors. All of the *trans* isomers were oxidized significantly slower than the esters of oleic, stearic, and palmitic acids. The results demonstrate that the heart mitochondrial β-oxidation system is markedly influenced by both positional and geometric isomerization of octadecenoic acid.

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LIPID COMPOSITION OF SWINE HEART PLASMA MEMBRANE. Barbara Babka and F.A. Kummerow, 205 Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, IL 61801.

Three groups of six pigs each were fed different fat diets. The control group was fed a 1% corn oil basal diet. The other two were given 10% test fat. The test fat was either corn oil or a 50% *trans* fat margarine stock. After 72 days all the animals were sacrificed; blood and heart samples were taken for lipid analysis. The plasma membrane was isolated from cardiac tissue and analyzed for phospholipid content via thin-layer chromatography and gas-liquid chromatography with the aid of a Hewlett Packard 5650 flame ionization gas chromatograph and 3380 A integrator. The differences in membrane component composition will be compared. The cholesterol content of blood and plasma membrane will also be compared.

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THE EFFECT OF *TRANS* FATS ON CALCIUM TRANSPORT IN THE ERYTHROCYTE. Dennis W. Koerner and F.A. Kummerow, 205 Burnsides Research Laboratory, University of Illinois, Urbana, IL 61801.

Dietary fats influence the composition of red blood cells. The effects of dietary octadecenoic *trans* fatty acids in the rat erythrocyte were examined. Time studies were carried out on the rate of *trans* acid incorporation at various dietary levels and their influence on the presence of other fatty acids. The effect of alteration on membrane fluidity was also measured. An analytical method of measuring calcium transport was developed and used. It was shown that increasing levels of *trans* acids in the membrane decreased the rate of calcium transport. An epidemiological survey comparing levels of *trans* fatty acids in the red blood cells of subjects from Japan, Romania, England, and the United States will be presented.

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THE EFFECT OF VEGETABLE AND PARTIALLY HYDROGENATED MARINE OILS ON THE MEMBRANE PHOSPHOLIPID COMPOSITION AND MITOCHONDRIAL FUNCTION OF HEART AND LIVER IN THE RAT. Rolf Blomstrand and Lennart Svensson, Dept., of Clin. Chem., Huddinge University Hospital, S-141 86 Huddinge, Sweden.

With the development of HPLC, and glass-capillary gas chromatography, it has been possible to study in more detail the metabolism of *trans* fatty acids. It is now recognized that partially

hydrogenated vegetable and marine oils contain a large range of positional isomeric fatty acids with *cis* and *trans* double bonds. Increased knowledge about the selectivity of acyltransferases that control the incorporation of dietary unsaturated *trans* fatty acids into membrane lipids is mandatory for the understanding of the influence of dietary fatty acids on the membrane function. We have shown earlier that rat heart cardiolipin showed a high affinity for erucic acid and that erucic acid to some extent replaced linoleic acid. It has been suggested that the decrease in the linoleic acid content of cardiolipin might influence the physical properties of the mitochondrial inner membrane with a concomitant loss of adenosine triphosphatase and phosphorilative activity. In the present work, we have studied the influence of partially hydrogenated marine oils and high erucic rapeseed oil on the ATP synthesis of isolated rat heart mitochondria and followed the incorporation of dietary *trans* unsaturated fatty acids in the phospholipids of the mitochondrial membranes. The incorporation into lecithin, phosphatidyl ethanolamine and cardiolipin has been particularly followed. ATP synthesis in isolated rat heart mitochondira was affected by the different dietary treatments. There was a small reduction of the ATP synthesis in the short-term experiment; however, after prolongation of the feeding time to 100 days, there was a general tendency to a depressed ATP synthesis in isolated rat heart mitochondria from rats fed rapeseed oil as well as partially hydrogenated marine oil, as compared with rats fed peanut oil. There were no effects of the different dietary oils on the ATP synthesis in isolated rat liver mitochondria.

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INFLUENCE OF DIETARY *TRANS* FATTY ACID ON ATHEROSCLEROSIS IN RABBITS. David Kritchevsky, Nancy A. Little, Larry M. Davidson, and Herbert Ruttenberg, The Wistar Institute, 36th St. at Spruce, Philadelphia, PA 19104.

Male, Dutch belted rabbits (8/group) were fed semipurified, cholesterol-free diets containing 14% fat. The diets contained 6% elaidic acid (H), 3.2% elaidic acid (L), or no *trans*-fatty acid (C). After 5 months rabbits were killed and plasma and liver lipids were analyzed. Activity of various hepatic enzymes was assayed, and aortas graded for severity of atherosclerosis. Plasma, erythrocyte membrane, and mitochondrial total fatty acids of rabbits fed diet H contained twice as much elaidic acid (13.2, 12.0 and 33.4 μg/mg protein) as did the same fractions from rabbits fed diet L (6.9, 4.8 and 18.7 μg/mg protein) ($p<0.001$). Plasma cholesterol, triglyceride and phospholipid levels (mg/dl) of groups H, L, and C were: 137, 114 and 47; 87, 39 and 31; and 69, 28 and 28, respectively. Activities (unit/mg protein) of glucose-6-phosphatase (microsomal) and fatty acid synthetase (cytosolic) in groups H, L, and C were: 2.7, 3.3 and 2.5; and 3.6, 3.6 and 3.2. Activities (H, L and C) of mitochondrial malate dehydrogenase were 1.1, 1.4, and 1.0; of β-hydroxybutyrate dehydrogenase, 6.7, 10.6, and 4.6; and of monamine oxidase, 8.7, 11.6, and 10.3. Only the difference between monamine oxidase activity in groups H and L was significant. Weight gain, liver weight, and average aortic atherosclerosis were virtually identical in the 3 groups. At the levels fed (3 to 6%), *trans*-fatty acids exerted no untoward effects. (Supported, in part, by grants HL 03299, HL 05209, and a Research Career Award HL 0734 from the NIH).

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ARSENIC LIPID BIOSYNTHESIS AND METABOLISM IN MARINE ORGANISMS. A.A. Benson, R.V. Cooney, and J.M. Herrera-Lasso, Scripps Institution of Oceanography, A-002, La Jolla, CA 92093.

Phosphate concentrations of surface waters approach those of arsenate in much of the ocean. Photosynthetic consumption in tropical waters reduces phosphate levels to nearly 10^{-8} molar. Being unable to discriminate between these similar ions, the algae of low-phosphate waters are faced with the problem of detoxicating natural arsenate. The process of reduction, successive methylation, and production of a non-toxic arsenophosphatide must have evolved in primordial oceanic algae in order to avoid accumulation of toxic intermediates, arsenite, methanearsonate, cacodylate, and trimethylarsine. Reaction of trimethylarsine with ubiquitous phosphoenolpyruvate yields trimethylarsoniumlactate, which is incorporated rapidly in phosphatidyltrimethylarsoniumlactate. This natural process is essential for survival of algae in the low-phosphate regions of the oceans. Food chain transfer of these arsenic compounds leads primarily to accumulation of trimethylarsoniumlactate itself. Arsenic content of marine products, therefore, consists largely of non-toxic trimethylarsonium derivatives and their biodegradation products. Biodegradation of the arsenophosphatide proceeds through the lysolipid and its deacylated product, glycerophosphoryltrimethylarsoniumlactate. Further degradation appears to involve oxidative attack to yield cacodylate from the trimethylarsonium group. Such oxidation is often mediated by superficial bacterial action on the algal membrane lipid. Bacterial metabolism appears to determine the chemical state and composition of arsenic compounds in seawater.

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THE PRESENCE AND METABOLISM OF STEROL-5,7-DIENES IN SOME MARINE ORGANISMS. L. John Goad, A. Sattar Khan and Richard M. Goodfellow, Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, United Kingdom.

Many marine invertebrate animals have now been analysed for their sterol composition and in most cases they contain complex mixtures of C_{27}-C_{30} sterols. A noteable feature of earlier reports on mollusc sterols was the frequent observation of $\Delta^{5,7}$-sterols but, with only a few exceptions, these sterol dienes were not identified. We have now reexamined the sterols isolated from species belonging to the Cephalopoda, Gastropoda and Pelecypoda, and found that $\Delta^{5,7}$-sterols were ubiquitous in these animals in amounts varying from 1 to 15% of the total mixture. In the bivalve *Mytilus edulis*, the major diene was identified as ergosterol but it was accompanied by a small amount of 7-dehydrocholesterol. Since molluscs constitute a major food source for many starfish (Echinodermata) which contain predominantly Δ^{7}-sterols, we have investigated the content, uptake, and metabolism of $\Delta^{5,7}$-sterols by *Asterias rubens*. Although radioactively labeled $\Delta^{5,7}$-sterol was absorbed by *A. rubens* during feeding upon a mollusc, the starfish was found to contain a very low concentration of $\Delta^{5,7}$-sterol in the total sterol mixture. Injected [4-^{14}C]-cholesta-5,7-dien-3β-ol was metabolized by *A. rubens* to yield 5α-cholest-7-en-3β-ol, the major sterol of this animal, but apparently it was not reduced to give cholesterol, although another metabolite was observed and tentatively identified.

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POLYUNSATURATED FATTY ACIDS OF PHYTOPLANKTER GLYCOLIPIDS. D.H. Beach and G.G. Holz, Jr., Department of Microbiology, S.U.N.Y., Upstate Medical Center, 766 Irving Avenue, Syracuse, NY 13210.

A comparison has been made of the fatty acyl groups of the monogalactosyldiglycerides of representative, cultured, photosynthetic, and marine flagellates. With few exceptions, the major components were methylene-interrupted, straight chain, C_{16}, C_{18} and C_{20} polyunsaturated fatty acids. They were distributed among the flagellate taxa as follows: Cryptophyta–18:3(9,12,15) and 18:4(6,9,12,15); Dinophyta–18:4(6,9,12,15) and 18:5(3,6,9,12,15); Heptophyta, Isochrysidales–18:3(9,12,15), 18:4(6,9,12,15) and 18:5(3,6,9,12,15); Haptophyta, Prymnesiales–18:4(6,9,12,15) and 20:5(5,8,11, 14,17); Chrysophyta, Eustigmatophyceae–20:5(5,8,11,14,17); Chlorophyta, Prasinophyceae–16:4(4,7,10,13), 18:3(9,12,15) and 18:4 (6,9,12,15); Chlorophyta Chlorophyceae–16:4(4,7,10,13) and 18:3(9,12,15). These findings support studies of some higher plants and algae indicating that photosynthesis-associated lipids are not acylated preferentially with α-linolenic acid, 18:3(9,12,15), and polyunsaturated C_{16} fatty acids. In one eustigmatophycean, *Nannochloris oculata*, monogalactosyldiglyceride 16:4(4,7,10,13) and 18:3(9,12, 15) each were only 0.2% of the total fatty acids, while 20:5(5,8,11, 14,17) was 72%.

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SEAWEEDS AS SOURCES OF NOVEL FATTY ACIDS. W.N. Ratnayake and J.S. Grossert, Dalhousie University, Halifax, Canada; R.G. Ackman, N.S. Technical College, P.O. Box 1000, Halifax, N.S. B3J 2X4, Canada.

Open-tubular gas-liquid chromatography (GLC) of fatty acids from eleven seaweeds, representing the phyla Chlorophyta, Rhodophyta, and Phaeophyta, revealed a total of twenty-two unusual components. Selected acids were isolated by combinations of preparative GLC and AG^{+}- thin-layer chromatography and then characterized by a combination of hydrogenation/GLC, partial hydrazine reduction, and oxidative ozonolysis. The results included five novel structures: 4-hexadecenoic; 13-hexadecenoic; 8,11-heptadecadienoic; 8,11,14-octadecatrienoic; and 8,11,14,17-octadecatetraenoic acids. An unusual fatty acid, *cis*-5, *cis*-11-octadecadienoic acid, initially identified from *Cladophora rupestris* (W.N. Ratnayake and R.G. Ackman, *Lipids 14*, 580 [1979]) was found as a minor component in all the eleven seaweeds. The new fatty acid *cis*-13-hexadecenoic (16:1ω3) and an unusual fatty acid *cis*-15-octadecenoic (18:1ω3) were isolated and identified from the seaweeds *Palmaria palmata* and *Halosaccion ramentaceum*, both belonging to the Rhodophyceae family. These are the only two seaweeds which showed a very high content (60 to 70%) of 5,8,11,14,17-eicosapentaenoic acid; therefore, there may be a close biochemical relationship between the C_{20} pentaene and the two "ω3" monoethylenic fatty acids. The C_{16} and C_{18} polyunsaturated fatty acids of *Agarum cribrosum* include the peculiar series with ethylenic bonds at ω1,ω4,ω7 and ω10 positions.

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FATTY ACID COMPOSITION OF SYMBIOTIC ZOOXANTHELLAE IN RELATION TO THEIR HOST. David G. Bishop and

Janette R. Kenrick, Plant Physiology Unit, CSIRO Division of Food Research, P.O. Box 52, North Ryde 2113, Australia.

Gymnodinoid dinoflagellate endosymbionts, commonly referred to as zooxanthellae, are widely distributed among marine invertebrates. They have been generally regarded as consisting of only one species, *Gymnodinium microadriaticum*, although this view has recently been questioned. In common with a number of axenically cultured dinoflagellates, the lipids of *G. microadriaticum*, isolated from the clam *Tridacna maxima*, have been shown to contain significant amounts of octadecapentaenoic acid, which is concentrated in the galactolipids of the chloroplast membrane. We have analyzed the fatty acid composition of the total lipids, the monogalactosyldiacylglycerols (MGG) and the digalactosyldiacylglycerols (DGG) of zooxanthellae isolated from eight species of corals, three species of clams, and a foraminiferan. Octadecapentaenoic acid was present in all samples, comprising up to 40% of the total fatty acids of MGG. The content of the acid in DGG was always lower than that in MGG. It was also apparent that the fatty acid composition of zooxanthellae MGG and DGG varied according to the host. This was exemplified by eicosapentaenoic acid, whose content in clam zooxanthellae MGG was less than 2% but in coral zooxanthellae MGG ranged from 9 to 22%. Corresponding values for the acid in DGG were 1 to 8% in clam zooxanthellae and 23 to 40% in coral zooxanthellae. The fatty acid composition of the zooxanthellae galactolipids of the foraminiferan were similar to those of the clams. The results suggest either that different species of zooxanthellae occur in corals and clams, or that the host is capable of exerting an effect on the fatty acid metabolism of the symbiont.

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STEROLS OF WILD AND CULTIVATED OYSTERS. Cathy J. Berenberg and Glenn W. Patterson,* Department of Botany, Patterson Hall, University of Maryland, College Park, MD 20742.

The sterol compositions of wild and cultured oysters, *Crassostrea virginica*, were examined. Wild oysters contained cholesterol (33%), brassicasterol (18%), 24-methylene cholesterol (21%), 22-dehydrocholesterol (6%), 24-nor-cholesta-5,22-dienol(4%), and several unidentified C-29 sterols. Cultured oysters were fed a defined diet of *Thalassiosira pseudonana* (90%) and *Isochrysis galbana* (10%). The sterol composition of these oysters revealed the predominance of cholesterol (19%), brassicasterol (21%), and 24-methylene cholesterol (58%). The dietary algae were cultured and their sterol compositions were analyzed by gas chromatography and mass spectroscopy. *Thalassiosira pseudonana* contained cholesterol (0.5%), brassicasterol (0.8%), 24-methylene cholesterol (77%), plus several unidentified C-28 and C-29 sterols. *Isochrysis galbana* contained cholesterol (1%) and brassicasterol (97%) with only traces of other sterols. Brassicasterol and 24-methylene cholesterol are obviously present in larger amounts in oysters fed algae containing those sterols whereas cholesterol is diminished in the cultured oysters. Nineteen percent of the sterol from cultured oysters was cholesterol, while less than 2% of the sterol in their diet was cholesterol; therefore, oysters must be able to bioconvert phytosterols to cholesterol, concentrate dietary cholesterol, or synthesize cholesterol *de novo*. Follow-up studies are in progress.

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EMULSIFIERS AS PROTEIN COMPLEXING AGENTS. E.J. Hughes, Food Industries, Ltd., Bromborough Port, Wirral, Merseyside L82 4SU, England.

Particularly in the baking industry for yeast-raised goods and cakes, and in the dairy industry for ice cream and synthetic cream products, interactions between emulsifiers and proteins are responsible for the favorable properties found in these applications. In yeast-raised goods the complexing ability of emulsifiers with flour proteins to improve proof tolerance, baking stability, and volume is mainly attributed to the anionic emulsifiers. These are the DATA ester derivatives of monoglycerides and sodium and calcium stearoyl-2-lactylate. Also in yeast-raised goods the more hydrophilic emulsifiers, e.g., polysorbate 60 and ethoxylated monoglycerides, are classified as dough conditioners due to their more polar nature for interaction with flour proteins. Various cake applications rely on the lowering of interfacial tension and the reinforcement of egg proteins by emulsifiers for emulsion and foam stability. Monoglyceride products and the α tending emulsifers, e.g., the propylene glycol esters, are particularly effective. During the manufacture of ice cream and synthetic cream type products, the fat particles agglomerate to form the desirable texture and overrun characteristics. The rate of agglomeration depends on the mixed emulsifier protein film around the fat particles which, in turn, depends on the structure and chemical properties of the emulsifier.

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ANALYSIS AND CLASSIFICATION OF EMULSIFIERS. Hans Brüschweiler, Swiss Federal Laboratory for Testing Materials (EMPA), CH-9001, Saint Gallen, Switzerland.

Emulsifiers consist of molecules with a lipophilic and a lipophobic (hydrophilic) part. They sustain the formation of emulsions. Food emulsifiers are mostly esters of edible fatty acids with polyalcohols. These emulsifiers are classified according to their polar part into carboxylates, sulfates and sulfonates, glycol-, glycerol-, sorbitan- and sugar esters of fatty acids. In order to analyze single emulsifiers, mixtures of emulsifiers, emulsifiers blended with other compounds, and emulsifiers extracted from food, they are preliminarily separated by liquid chromatography, HPLC, or thin-layer chromatography. The emulsifier constituents of different polarities obtained are hydrolyzed with alcoholic KOH solution. After evaporation of the solvent and silylation, GC determinations of the silylated components (carboxylic, dicarboxylic, hydroxycarboxylic acids, glycols, polyglycols, glycerol, polyglycerols, sorbitol derivatives, mono- and disaccharides, etc.) are carried out.

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HPLC-ANALYSIS OF NONIONIC EMULSIFIERS. John Sørensen, Grindstedvaerket A/S, Edwin Rahrsvej 38, DK-8220 Brabrand, Denmark.

Monoglycerides are commonly used emulsifiers in processed foods containing fats and oils (e.g., margarine, shortening, ice cream, and bakery products). Fatty acid esters of other polyols (e.g., propyleneglycol, sorbitan, and sucrose), and monoglyceride derivatives such as acetic acid, lactic acid, and diacetyl tartaric acid esters are also used. With the appearance of HPLC (high performance liquid chromatography), we have acquired a valuable supplement to the analysis methods traditionally used for the determination of emulsifiers. With acetic acid esters of monoglyceride as an example, some of the possibilities available when employing HPLC in the emulsifier analysis are shown. By partial acetylation of a distilled monoglyceride, five components are obtained if no distinction is made between the long chain fatty acids involved. The five components are monoglyceride, monoacetylated monoglyceride, diacetylated monoglyceride, diglyceride, and acetylated diglyceride. A method used for quantitative determinations of these is described. The separation is obtained by using gradient elution on a Lichrosorb 10 Diol column and UV-detection at 220 nm. A linear of 2-propanol in isooctane has been used. Other examples of HPLC-emulsifier analyses are shown.

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USE OF POLYGLYCEROL ESTERS IN FOODS. Russell T. McIntyre and Karen Hendrix, Capital City Products Co., Division of Stokely-Van Camp, Inc., Columbus, OH.

Polyglycerol esters (PGE) are used in broad areas of application. They may be prepared to cover the HLB range from about 3 to over 12. Liquid or solid products may be manufactured. Solid esters are most often used as emulsifiers when whipped products are made; products containing the liquid oleate emulsifiers are employed when aeration is not required. Combinations of the two types may be used for controlled shipping as in icings. Polyglycerol esters have a variety of uses in the food and beverage industries. PGEs are employed in dietary foods such as cakes, creams, margarines, toppings, ice cream and others. PGE's ability to act as a crystallization inhibitor is useful in the salad oil industry. In the beverage industry, the PGE's have been used as clouding agents in flavored drinks. In the pharmaceutical industry, polyglycerol esters may be used in the treatment of malabsorption syndromes as bile salt replacement. The laurate esters exhibit bacteriostatic activity. Lotions and creams, in both the pharmaceutical and cosmetic industries, may contain PGE's.

143B

SURFACE ACTIVE PROPERTIES AND ASSOCIATE STRUCTURES OF POLYGLYCEROL ESTERS IN FOOD EMULSIONS. Wil Hemker, SOM Durkee Foods; Dwight P. Joyce Research Center, 16651 Sprague Road, Strongsville, OH 44136.

Liquid crystalline types and the crystalline gel state of tri-, octa-, and decaglycerol esters are identified. The behavior of crystalline and liquid crystalline forms of these polyglycerol esters were studied in oil and water emulsion and model food systems. Monostearate esters of the triglycerol and octaglycerol polymers exhibit liquid crystalline behavior of hexagonal II and lamellar type, respectively. Additionally, both esters form metastable α gels. Triglycerol monoester, primarily a mixture of palmitate and oleate, and octaglycerol monoleate do not show liquid crystalline activity but form stable α crystalline gels. The lamellar liquid crystalline and α gel forms of octaglycerol monostearate assist in providing interfacial structure, to enable the formation of freeze-thaw stable (oil-in-water) o/w emulsions. Polyglycerol esters forming stable α gels modify the texture in lipid/protein systems and provide structure and surface activity to form stable water-in-oil (w/o) emulsions.

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MULTIPLE EMULSIONS: SOME ASPECTS OF THEIR DEVELOPMENT AS DRUG DELIVERY SYSTEMS. Sylvan G. Frank, College of Pharmacy, The Ohio State University, 500 W. 12th Ave., Colum-

bus, OH 43210.

Oil/water/oil and water/oil/water emulsions are being studied as potential delivery systems for the controlled or prolonged release of drugs that have a short biological half-life. If drug is either dissolved or suspended primarily within the innermost phase, it will be forced to cross several interfacial barriers and pass through several phases prior to release. Multiple emulsions can form at the point of inversion of a primary emulsion, or by the addition of a primary emulsion to a suitable immiscible phase. By the latter method, either O/W/O or W/O/W systems can be formed depending upon the phase volume ratios, the nature and concentration of the surfactants, and in certain cases, the method of preparation. The in vitro release of drug can be significantly influenced by the relative surfactant concentrations in multiple emulsions stabilized by different surfactants in the oil and water phases. Likewise, if the nature of the interfacial barriers is altered by the presence of additives, the rate of release can be changed. In addition to controlling drug release, the nature of the interfacial barriers can also influence stability, the most stable systems being those in which an apparent liquid crystalline phase exists. Such systems show enhanced stability under centrifugation when compared to systems in which no liquid crystalline phase is present. Under these conditions, internal droplets can be observed to sediment within the larger droplets as the larger droplets themselves undergo sedimentation. Sedimentation of the internal droplets and in vitro drug release can be retarded by increasing the viscosity of the water phases, and this effect can be observed even at high phase volume ratios of the internal disperse phase.

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TECHNIQUES IN THE ISOLATION AND IDENTIFICATION OF STEROIDS OF INSECTS AND ALGAE. Malcolm J. Thompson, Glenn W. Patterson, Samson R. Dutky, James A. Svoboda, and John N. Kaplanis, Insect Physiology Lab., Bldg. 467, BARC-East, Beltsville, MD 20705.

Isolation and identification techniques required for studying the metabolism and pathways of steroids in insects and algae have been well developed. Our laboratories have employed specific extraction procedures for the isolation and separation of sterols from crude extracts of insects and algae. Once the sterols are isolated, various methods such as adsorption and argentation column chromatography are employed to separate the individual sterols. For positive identification of the individual sterols we have employed a combination of analytical tools such as: gas-liquid chromatography (GLC) on 3 or 4 different columns for tentative structural assignment; ultraviolet, infrared (IR); nuclear magnetic resonance (NMR); and mass spectral (MS) analyses for confirmation of structure. These methods will be discussed in light of their significance or enhancement of the identification of steroids. We have found, for example, with the exception of sterols isomeric at C-24, that all of the more than 90 sterols identified by us could be distinguished from each other on the basis of GLC retention times. By using double bond, steric and alkyl substituent separation factors of sterols, previously unidentified sterols could be tentatively identified without the usual spectroscopic methods of identification. The C-24 isomeric sterols could be differentiated by NMR spectroscopy at 100 and 220 MHz. NMR spectra now can be used to determine whether algal sterol with an alkyl substituent at C-24 has the α- or β-configuration at C-24 or consists of an epimeric mixture. The orientation and location of hydroxyl groups of the steroidal insect molting hormones are readily established by NMR spectroscopy. Infrared analyses indicate whether sterols contain a Δ^5, a *trans* Δ^{22}-bond, or terminal methylene group and whether a 24-ethylidene group is *cis* or *trans*. Mass spectral analyses verify the position of these double bonds and give a wealth of specific analytical and structural information that enhance the identification of steroids of insects and algae.

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HIGH RESOLUTION GLC ANALYSIS OF STEROID HORMONE METABOLITES WITH CHEMICALLY BONDED STATIONARY PHASE GLASS CAPILLARY COLUMNS. C. Madani and E.M. Chambaz,* Université Scientifique et Médicale, Biochimie Endocrinienne, CERMO, B.P. 53 X 38041, Grenoble Cedex, France.

A new approach has been developed for the manufacture of capillary columns with stationary phases chemically bonded to the glass surface. The method involves: (1) synthesis of *reactive siloxane polymers* from variously substituted dichlorosilanes—homologous and/or heterologous polymerization can result in a wide range of polymer polarity; (2) treatment of the *glass surface* (HCl); and (3) *coupling* of the reactive polysiloxane to the glass surface under proper conditions. Apolar (e.g., dimethyl polysiloxane) and polar (e.g., diphenyl-polysiloxanes) phases have been readily obtained. The approach appeared highly flexible since stationary phases of controlled polarity could be obtained by mixed substitutions (e.g., methyl-phenyl, methyl-phenyl-cyanopropyl polysiloxanes). The method is also reproducible and quite inexpensive. The resulting high resolution chromatographic systems proved to be well suited to biochemical analysis under repeated temperature programming conditions. They have been used routinely over the past three years, mostly for qualitative and quantitative study of steroid metabolites in human.

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STEROL ANALYSIS FOR THE DETECTION OF ADULTERATION OF MILK FAT. Elfriede Homberg and A. Seher,* Federal Centre for Lipid Research, Piusallee 68-76, D-4400 Muenster, Germany.

Gas chromatographic analysis of the sterol composition of butterfat allows detection of an adulteration with vegetable fat by the identification and quantification of sitosterol. Pure butterfat does not contain sitosterol and campesterol, but does contain in small quantities a substance with a retention time similar to campesterol. Besides cholesterol, which amounts to nearly 99% of the total sterols, the following sterols could be identified: Δ5,7-cholestadien-3β-ol, Δ7-cholesten-3-on, Δ5-cholesten-3-on, and Δ4,7-cholestadien-3-on. To quantify the amount of vegetable fat added to butterfat, one has to take into account: (1) the total sterol content of the sample and the sitosterol concentration in the sterol mixture; and (2) the average values for total sterols and sitosterol in pure butterfat and vegetable fat. To partially conceal an adulteration of butterfat, cholesterol was added since it diminishes the concentration of sitosterol in the sterol mixture of the sample, but increases the amount of total sterols. Thus, determination of total sterol content also indicates this kind of adulteration.

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SELECTIVE REACTIONS IN THE ANALYSIS AND CHARACTERIZATION OF STEROIDS BY GC-MS. Charles J.W. Brooks, W. John Cole, and Helen B. McIntyre, Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ, United Kingdom.

Gas chromatography-mass spectrometry (GC-MS) is a powerful technique for the analysis and characterization of steroids. Its efficacy is limited, nevertheless, because steroid mixtures—whether of natural origin only, or augmented by synthetic analogs—often contain closely similar components that are poorly distinguished. The fortuitous overlap of gas chromatographic peaks from disparate compounds also impairs the definition of retention data. Controlled modification of the sample by means of selective reactions is therefore a valuable adjuct to the application of GC-MS. Two examples will be discussed: (1) The enzyme cholesterol oxidase, isolated from various micro-organisms, catalyses the oxidation of many 3β-hydroxy-5-enes (with concomitant isomerization) to 4-en-3-ones; 3β-hydroxy-5α-steroids are also oxidized to the corresponding 3-ones, but other steroids (3α-hydroxy- or 5β-isomers, etc.) are unaffected. The mild conditions required (pH7, 30 C) are advantageous for the analysis of sensitive steroids, and the retention index increments, as well as the mass spectra of the ketones, are characteristic. (2) Steroids possessing 1,2-diol or 1,3-diol groupings are exemplified by estriols, 2-hydroxy-estrone, 20,22-dihydroxy-cholesterols and many corticosteroids. The selective formation of cyclic derivatives can provide several analytically useful features such as conventient retention times, moderate mass increments (24 amu for a methaneboronate), distinctive mass spectra, and usually abundant molecular ions.

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ANALYSIS OF CHOLESTEROL OXIDE AND ITS METABOLITES IN MOUSE TISSUES. Homer S. Black, Photobiology Laboratory Bldg. 203, Rm. 118, Veterans Admin. Hospital, Houston, TX 77211.

The presence of cholestan-3β, 5α, 6α-epoxide—an oxidation product of cholesterol—has been demonstrated in a variety of animal tissues. No definitive biologic role for this sterol has been forthcoming, although the physiologic implications of its occurrence are many. The presence of the epoxide in ultraviolet light (UV)-irradiated skin was detected by a combination of thin-layer (TLC) and gas-liquid radiochromatographic techniques in 1971 and led to the suggestion that it might be responsible for the carcinogenic properties of UV. Subsequently, *in vivo* levels of this sterol in skin of UV-irradiated animals were quantitated by gas-liquid radiochromatography, after TLC separation and preparation of the radiolabeled acetate ester. Presence of the compound in mouse liver was detected by gas-liquid chromatography of the trimethysilyl ether and confirmed by mass spectrometry. In all cases, however, *in vivo* quantitations were complicated by substrate induction of sterol epoxide hydrase, an enzyme that hydrates the epoxide to form cholestan-3β, 5α, 6β-triol (triol). Consequently, the relation of the sterol epoxide to UV-carcinogenesis is, as yet, unclear, and recent studies suggest that if this sterol is involved, then further metabolism of the compound must be involved. A practical means of examining the metabolites of the epoxide was developed employing thin-layer radiochromatographic scanning. Using this procedure the relationships of substrate concentration, time of reaction, and pH optimum to reaction rate were determined. Although in short

term *in vitro* incubations of liver homogenates, the bulk of epoxide is hydrated to triol, at least eight other metabolites are detectable by TLC autoradiography. These metabolites are readily separated by programmed multiple development of high performance TLC plates and are currently being studied for biologic activity.

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ANALYSIS AND STRUCTURE DETERMINATION OF UNSATURATED BILE ACIDS. A. Kuksis and P. Child, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Canada M5G 1L6.

Unsaturated cholanoic acids are known to occur as artifacts of certain chemical derivatization processes, during high temperature gas-liquid chromatography (GLC), and as intermediates in mass spectrometry (MS) of saturated bile acids. Of particular interest are reports of their occurrence in natural bile acid extracts under conditions where their artifactual formation is unlikely. Because structural identification of such compounds is often complicated by a lack of knowledge of their analytical properties, a series of monounsaturated cholanoic acids with double bonds in rings A, B, and C were prepared by $POCl_3$ and $ZnCl_2$ dehydration of saturated bile acids with selectively blocked hydroxyl functions. The synthetic 5β-cholenoic acids obeyed the general rules of chromatographic mobility based on the overall shape of the molecule and the number and configuration of the functional groups. The cholenoic acids were indistinguishable from their saturated analogues on thin-layer chromatography (TLC) on plain silica gel, but those compounds with sterically exposed double bonds were resolvable by $AgNo_3$-TLC using chloroform-methanol solvent systems. Constant retention factors attributable to the double bond were observed for all of the double bond types on several packed-column GLC systems. In many cases, the retention time differences were large enough to allow baseline resolution of the unsaturated compounds and their saturated counterparts. For the remaining types, the small, but real, retention increments were exploited on capillary GLC systems, allowing an improved resolution on polar columns. Several characteristic fragments were observed in the mass spectra, which in conjunction with the chromatographic properties, permitted an unambiguous distinction between monounsaturated acids, and between saturated and unsaturated bile acids of the same number and configuration of functional groups. For complete structural identification of saturated and unsaturated bile acids, capillary GLC-MS represents the ideal state of the art, but the less expensive combination of $AgNo_3$-TLC and GLC also can yield much useful information concerning the structure of naturally occurring 5β-cholenoic acids. (Supported by Ontario Heart Foundation and Medical Research Council of Canada.)

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THE DETERMINATION OF STEROIDS WITH AND WITHOUT NATURAL ELECTROPHORES BY GAS CHROMATOGRAPHY AND ELECTRON CAPTURE DETECTION. Colin F. Poole and Albert Zlatkis, University of Houston, Chemistry Department, Houston, TX 77004; E. David Morgan, Department of Chemistry, University of Keele, Keele, Staffordshire ST5 5BG, Great Britain.

The electron-capture detector (ECD) is the most sensitive of the selective gas chromatography detectors with a maximum response for a rather ill-defined group of organic substances containing halogen atoms, nitro groups, or conjugated unsaturated systems. With the exception of a few steroids (those containing conjugated ketone groups when the ECD is used for their analysis), derivatization is required to improve their thermal and chromatographic properties prior to gas chromatography. Two types of derivatizing reactions can be envisaged: in one case the derivative is formed to improve the chromatographic properties of the steroid and advantage is taken of the natural electron-capturing properties of the steroid for its detection; in the second case the derivatizing reagent is used both to improve the chromatographic properties and to introduce an electron-capturing tag for its detection into the steroid. The former case will be illustrated by the determination of melengestrol and the ecdysones. The insect moulting hormones (ecdysones), after formation of their trimethylsilyl ethers, can be determined with an ECD throughout the complete life-cycle of the desert locust from egg to adult. For the latter case, the haloacyl esters, flophemesyl ethers, t–buflophemesyl ethers, pentafluorobenzyloximes, and pentafluorophenylhydrazone derivatives can be formed with steorids. For the particular case ofthe corticosteroids, the side chain can be selectively derivatized with halogen-substituted aromatic boronic acids, and the derivatives detected with the electron-capture detector.

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ON DERIVATIVES OF OXOALCOHOLS OBTAINED BY HYDROCARBOXYMETHYLATION OF INTERNAL OLEFINS. H.H. Maag, Chemische Werke Hüls AG, Postfach 13 20, D-4370 Marl 1, West Germany.

Primary fatty alcohols are prepared by hydrogenation of natural fatty acids (or methylesters) by a modified Ziegler synthesis and by oxo-reaction of α-olefins. The carbonylation reaction leads to primary alcohols with partial branching. Internal olefins, which are obtained by dehydrogenation of n-paraffins or dehydrochorination of monochloroalcanes, yield primary fatty alcohols with high branching when undergoing the normal oxo-process. However, when internal olefins are submitted to hydrocarboxymethylation, fatty acid methylesters with high linearity are obtained. These can be hydrogenated to primary alcohols with high linearity. Surfactants based on these alcohols, i.e., ethoxylates and ethersulfates, have been tested in comparison to known alcohol derivatives as to detergency, wettability, foaming ability, and several other characteristics important for their application in detergents and cleaning agents. Ecological data such as biodegradability and fish toxicity were determined as well.

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ENERGY CONSUMPTION IN THE COURSE OF MANUFACTURING AND USE OF DETERGENT BUILDERS. H.D. Nielen and J. Kandler,* Hoechst A.G., Werk Knapsack, 5030 Hürth-Knapsack, West Germany.

There is worldwide discussion on problems that are connected with the increasing demand of energy. Obviously these questions are also of great interest to the producers of detergents and raw materials, and to the consumers. Figures are given with respect to the energy consumption in households. Especially in Western Europe, there is a wide variety of washing processes which differ from each other with respect to energy consumption. Possibilities of saving energy are given. Modifications of the washing process may require reformulation of the detergents. Possibilities and limits are indicated with special regard to detergent builders. Results of washing tests covering the mentioned items are imparted. The energy demand for manufacturing individual detergent builders is analyzed.

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CHLORINATED SURFACTANTS. Roger Perron and Josiane Petit, Centre National de la Recherche Scientifique, 2-8 Rue Henry Dunant, 94320 Thiais, France.

Radical chlorination of fatty acids is first described. It is shown that sodium or triethanclamine salts of chlorinated fatty acids are soluble in water or in oil only for low chlorine contents. They are unstable and slowly transform into polyesters and lactones. Various substitution reactions were attempted in order to ameliorate water solubilities and emulgator properties, while an ester of a polyoxyethylene methyl ether was prepared. Limited chlorination was also applied to α-sulfonic fatty acids, and various esters and salts derivatives were obtained and studied. Superficial and interfacial tensions were measured as functions of concentration for the different products, and the ability to give emulsions and microemulsions is discussed.

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NATURAL FATTY ALCOHOLS: THEIR COMEBACK AS DETERGENT RAW MATERIAL. A REVIEW OF PRODUCTION TECHNOLOGY. M. Ballestra, Ballestra SpA, Via G Fantoli, 21/17, 20138 Milano, Italy.

Pretreatment of fats and oils prior to high pressure hydrogenation are described in detail. Hydrogenolysis, either to produce fatty alcohols directly from triglycerides, methylesters, pre-esterified fatty acids, or fatty acids is described. Newer sulphonation methods to sulphate fatty alcohols and their derivatives are dealt with. Industrial process data are given for the processes described as well as specifications of end products. Also economic aspects are dealt with. Other natural fat derived surfactant raw materials and their transformation into surfactants are also briefly mentioned.

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ENZYMES FOR LOW TEMPERATURE WASHING. M. Hilmer Nielsen, Novo Industri A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark.

The increasing use of synthetic fibers that are damaged by temperatures above 50 to 60°C has switched the washing habits in Europe in the past 5 to 10 years toward the use of lower washing temperatures. Furthermore, the energy crisis has focused interest upon washing at ambient temperatures for the purpose of saving energy. In order to compensate for the lower washing efficiency at decreased temperatures, enzyme producers have devoted much R & D capacity to screen for new proteolytic enzymes that are more suitable for the washing at lower temperatures. The result of screening program carried out for several years, which used a new detergent protease with interesting characteristics, will be presented.

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SOAPS AND DETERGENTS AND THE ENVIRONMENT. A. Taylor, Corporate Technical Manager, Albright & Wilson Limited, P.O. Box 15, Whitehaven, Cumbria CA28 9QQ England.

For more than a quarter of a century there has been a constantly growing awareness of, and interest in, the effects of the detergent industry, by the nature and use of its products on the environment.

This interest has largely been in terms of the aquatic environment, but also, as with other industries, in terms of other socioecological issues. This paper concentrates on a review of detergents and their components, both organic and inorganic, and their impact on the aquatic environment in different parts of the world. It deals in particular with sodium tripolyphosphate, a major inorganic component in detergents, and presents an up-to-date and critical review of technical, environmental, sociological and legislative aspects of this product on a worldwide basis and a comparison of it with some of its proposed partial substitutes.

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INTRODUCTION: USE OF LECITHIN, CHOLINE, AND OTHER NUTRIENTS TO MODIFY BRAIN FUNCTIONS. Richard J. Wurtman, Massachusetts Institute of Technology, 56-245 Cambridge, MA 02139.

The functional activity of the brain depends on the transmission of chemical signals, mediated by neurotransmitters, from each neuron to the 1,000-or-so other neurons with which it communicates. About 30-40 compounds have been identified that probably function as neurotransmitters somewhere in the central nervous system. The rates at which several of these compounds are synthesized (and, consequently, the amounts of them that are released each time a neuron "fires") has been shown to depend upon the amounts of certain nutrients that are available to them. The key nutrients are compounds like choline (from lecithin), tryptophan, and tyrosine, which are transformed within the neuron to the neurotransmitters acetylcholine, serotonin, dopamine, and norepinephrine. This capacity of dietary constituents to increase neurotransmitter production, and thereby to affect the transmission of signals within the brain, now provides the basis for a new way of treating various brain diseases. This talk will describe in more detail how one goes about showing that a particular compound (e.g., acetylcholine) is a neurotransmitter, that its synthesis and release can be affected by nutrients, and that the use of nutrients in this manner can be effective in treating certain diseases.

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PRECURSOR CONTROL OF ACETYLCHOLINE SYNTHESIS. Steven H. Zeisel, Massachusetts Institute of Technology, 56-245, Cambridge, MA 02139.

Synthesis of the neurotransmitter acetylcholine can be influenced by manipulation of the availability of choline to the brain. Dietary intake of choline molecules, primarily in the form of lecithin, elevates plasma choline concentration. This elevation increases net choline transported into brain via a carrier mechanism in the blood brain barrier. The intraneuronal enzyme choline acetyltransferase is unsaturated at normal brain choline concentrations, and whenever brain choline is increased it synthesizes more acetylcholine. In rats, choline administration in the diet, or by injection, increases acetylcholine levels in all regions of brain. Post-synaptic activation of tyrosine hydroxylase and post-synaptic increases in dopamine metabolites demonstrate that release, as well as synthesis, of acetylcholine is accelerated. Actual measurement of choline-induced increase in release of acetylcholine molecules can be accomplished in preparations of phrenic nerve-diaphragm, or of isolated perfused heart. Lecithin administration also increases brain acetylcholine synthesis, avoids intestional degradation of choline molecules to trimethylamine, and provides a more prolonged elevation in plasma choline concentration. For this reason it is the preferred method for therapeutic administration of choline molecules.

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METABOLIC FATE OF DIETARY LECITHIN. U. M. T. Houtsmuller, Unilever Research Vlaardingen, The Netherlands.

Although every mammalian cell is able to synthesize phosphatidylcholine (PC), specific health-promoting effects have been ascribed to dietary lecithin for a long time. Recently, orally ingested lecithin has been implicated in the alleviation of certain neuropathological conditions by raising the free choline levels of plasma and brain tissue. In many of these studies, however, crude preparations of lecithin have been used, complicating the interpretation of the results. In this paper the metabolism of dietary PC will be discussed with a view to plasma-free choline levels. In the intestinal lumen, lyso-PC is formed, which is partly reacylated in the enterocyte or further degraded to its constituents. Reacylated PC is used mainly for chylomicron formation. During metabolism of the latter in plasma, part of the lecithin is transferred to other lipoproteins, particularly HDL. By way of direct exchange with cells, conversion to lyso lecithin by LCAT, or endocytosis of LDL, the lipoprotein lecithin is taken up in tissue cells. After degradation the choline part may reenter the bloodstream. Quantitative aspects of these processes will be discussed.

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CLINICAL USES AND METABOLIC EFFECTS OF EXOGENOUS LECITHINS. John H. Growdon, Tufts-New England Medical Center Hospital, Boston, MA 02111.

Lecithin adminstration increases blood choline, brain choline, and brain acetylcholine levels in rats; if a similar sequence occurred in humans, lecithin administration might be an effective mode of therapy for brain diseases associated with deficient acetylcholine neurotransmission. In order to determine the effects of ingesting common foods on plasma choline levels, we gave six normal subjects meals that contained very little lecithin (less than 100 mg of choline base/day) and meals that contained large amounts of lecithin (about 650 mg of choline base/day). Plasma choline levels did not vary during the low-lecithin diet, whereas they doubled during consumption of high-lecithin meals. On separate days, the same subjects consumed a single dose of 20 g of soy or egg lecithin (both about 80% phosphatidylcholine) in addition to the low-lecithin diet. Soy and egg lecithins were equally effective in raising plasma choline levels 400% above fasting values within 4 hours. Lecithin ingestion did not alter blood pressure, pulse rate, or hormone levels; plasma cholesterol levels did fall, however. The ability of lecithin administration to increase plasma choline levels in humans led to its use in treating disease characterized by deficient acetylcholine release, such as the choreic facial movements (tardive dyskinesia) that occur in some patients who take antipsychotic drugs. We gave 20-40 g/day of lecithin (55-80% phosphatidylcholine) to 7 patients with tardive dyskinesia and found that the movements decreased in all 7. We have just begun to test the effects of lecithin administration in other diseases, such as mania, age-related memory loss, and even Alzheimer-senile dementia. These clinical trials illustrate a new form of medical therapy, in which a naturally-occuring dietary constituent (lecithin) is prescribed as a drug to increase brain levels of a neurotransmitter (acetylcholine) and to treat psychiatric and neurologic disorders.

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INTERACTION OF FAT-CONTAINING FOOD WITH PLASTIC PACKAGING. C.G. vom Bruck, K. Figge, and F. Rudolph, Unilever Forschungsgesellschaft mbH, Behringstr. 154, 2000 Hamburg 50, Germany.

The interaction of food ingredients with packaging material can have two consequences: (1) The food ingredient changes the properties of the plastic in such a way that it no longer fulfills the protecting function, i.e., that the plastic may become brittle or crack under stress. This is a problem of suitability and private contracting. (2) The interaction of the food ingredient with a plastic will normally increase the migration of packaging ingredients into the food. This is an unwanted effect and can have legal consequences as well. One extreme is a plastic like PVC where there is practically no interaction with fatty food. Triglycerides can penetrate into less dense plastics in measurable amounts, increasing the diffusion coefficients of the additives by changing the polymer structure. In the case of polyethylene or polypropylene, this interaction is not accompanied by a change of volume of the polymer, whereas in the case of certain polystyrenes the uptake of fat is accompanied by an increase of volume of the polymer, thus resulting in the tendency to cracking under stress. Detailed measurements by K. Figge on these systems will be discussed. A mathematical model by F. Rudolph was successfully applied to calculate the migration of additives, provided the diffusion coefficients and Nernst partition coefficients of the components considered are known at the temperature they are used. Because of this predictable behavior, legal systems covering the use of plastics for food packaging can be based on additive limits in the plastic material as well as on migration limits. For fatty food containing water and proteins or carbohydrates the interaction with plastic material will depend on the food's composition and structure. Results obtained with a newly developed test procedure to characterize such food will be presented, and the consequences on legal systems based on specific migration limits will be discussed.

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CATALYSIS OF LIPID OXIDATION BY POLYOLEFIN PACKAGING MATERIALS. S.G. Gilbert, J.A.F. Faria, and C. Mannheim, Department of Food Science, Rutgers State University, P.O. Box 231, New Brunswick, NJ 08903.

The increase in polyolefin packaging for lipidogenous foods requires consideration of the potential catalysis of oxidation by promoters formed by prior oxidation of residual olefenic groups in the polymer. The existence of tertiary carbon structure in low density polyethylene and polypropylene also can contribute to pro-oxidation. In this manner hydroperoxides and other partly reduced oxygen species (PROS) can be created in the fabrication of packages during extrusion and treatment by corona discharge. A new procedure has been developed for measuring the degree to which such materials can influence lipid oxidation on contact which simulates the food/polymer interface. The method and data on some such systems will be presented.

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THE EFFECTS OF PROCESSING, PACKAGING AND STORAGE ON THE OXIDATIVE STABILITY OF FOOD SYSTEMS. Jack R. Giacin, School of Packaging, Michigan State Univer-

sity, East Lansing, MI 48824; J. Ian Gray, Department of Food Science & Human Nutrition, Michigan State University.

The nutritional quality of both fresh and processed foods depends to an appreciable extent upon the processing conditions and protection provided by packaging. In the selection of an appropriate packaging system for food packaging applications, there are a number of factors that must be considered, which include: (1) the stability of the foodstuff, which may involve the reaction of specific food components such as lipids, protein, and certain vitamins to oxygen and changes in the water activity of the product. The stability of the foodstuff will be a function of the chemical, biochemical, and physical nature of the product, and will be influenced markedly by the permeability or barrier properties of the package; (2) the prevailing environmental conditions to which the product is exposed during distribution and storage. Such environmental conditions as temperature and relative humidity will dictate the barrier properties required of the packaging system (3); the compatability of the package with the method of preservation selected. In this paper, the authors delineate the environmental factors that contribute to the oxidation of food components in selected food systems and discuss packaging and storage conditions for minimizing the extent of product quality loss due to oxidative reactions.

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COCOA BUTTER SUBSTITUTES FROM MANGO FAT. B.P. Baliga and A.D. Shitole, M/S. Tata Oil Mills Company Limited, Sewri, Bombay-400 033, India.

Mango fat obtained by solvent extraction of the kernels of the mango fruit (Magnifera indica) has been studied for its suitability for making cocoa butter substitutes. The fat has been fractionated from acetone at low temperatures in one and/or two stages in order to segregate suitable solid fractions having physical properties closer to cocoa butter. The data pertaining to the solidification characteristics and dilatometric behavior of the mango fat, its acetone-fractionated products, and their admixtures with cocoa butter in equal proportions have been determined in order to assess their compatibility with cocoa butter. Fractionated mango fat can serve as a good substitute for cocoa butter.

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QUALITY OF COCOA BUTTER. H.R. Kattenberg, Cacaofabriek de Zaan B.V. Postbus 2, Koog Aan de Zaan, Holland.

Cocoa butter is an important and expensive raw material for the chocolate and confectionary industry. It must therefore fulfill high quality standards including taste, melting, and crystallising behavior and chemical composition. As a manufacturer of cocoa butter, one can control the quality by proper selection and blending of the cocoa beans and controlling the processing of the beans to cocoa butter. Selection criteria for the beans are based on country of origin and quality of the beans such as size, flavor, and amount of damage. Processing of the beans includes breaking, winnowing, roasting, alkalizing, grinding, pressing, filtration, and deodorization of cocoa butter. The quality assessment is done in the laboratory using a number of physical and chemical analyses. Melting characteristics are examined by pulse NMR and DSC, and crystallising properties by cooling curves and thermo-rheology. Chemical composition is examined by the determination of the usual characteristics such as IV, FFA, PV, etc., and by glyceride analysis. In this paper the impact of origin and quality of the beans and processing on the quality of cocoa butter is in particular discussed.

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THE EPOXYSTEARIC ACID CONTENT OF SALSEED FAT. T.W. Hammonds and R.V. Harris, Tropical Products Institute, Industrial Development Department, Culham, Abingdon, Oxon OX14 3DA, England.

The Sal tree (*Shorea robusta*) grows extensively in the forest regions of India. About 200,000 tons of seed are collected annually, and the extracted fat used traditionally for local soap manufacture. Recent work in India and elsewhere has shown that the seed fat can be extracted, refined, and fractionated to give a 70% yield of solid fat that has potential as a cocoa butter substitute. In view of the uncertainty surrounding the epoxystearic acid content of sal fat, a survey has been carried out on the levels of this unusual fatty acid in the fat of sal seed collected at various sites in India. Fat samples, extracted from seeds obtained at different stages of the processing sequence–harvesting, drying, dewinging, and storage–have been examined and the levels of epoxystearic acid determined, in an attempt to throw some light on its origin, and, if possible, to suggest harvesting and processing procedures which would limit its formation. The results of the survey will be presented and discussed.

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A BIBLIOGRAPHY ON THE COMPOSITION, PROCESSING, QUALITY AND USES OF SOYBEAN OIL. John C. Cowan, Department of Chemistry, Olin Hall, Bradley University, Peoria, IL 61625.

The data bases of AGRICOLA (National Agricultural Library), CA (Chemical Abstracts) Search, and FSTA (Food Science and Technology Abstracts) were searched from their dates of inception (1970, 1967, and 1970 respectively) to July 1979 for references to edible soybean oil and related subjects. Only citations relative to the composition, processing, refining, quality, conversion to edible or pharmaceutical products, and uses of soybean oil and related subjects were sought. Many unneeded references as well as duplications were obtained in an attempt to find all pertinent publications. Emphasis was placed on flavor, flavor stability, and dietary use as related to oil quality. Some data bases did not have all articles that contained information on soybean oil keyworded under either soybean or vegetable oil; thus, some desirable references were missed initially. Examples of search problems will be given. Each selected abstract was read and appropriate keywords assigned. Each word was given an eight or less character code for key punching. Each reference and code were entered into the computer at the Northern Regional Research Center. The bibliography includes an introduction, lists of references in alphabetical order by senior author and chronoligically by chemical abstract citation within selected time frames, and a subject and author index. The computer printout was used as the master copy for printing. It is planned to publish not only the complete bibliography but selected lists of references on specific subjects, such as processing, oxidation, and antioxidants, separately.

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TRIGLYCERIDE STRUCTURE OF NAKED SEEDED PUMPKIN SEED OIL (*Cucurbita pepo*, VAR. LADY GODIVA). J. Mark Black, Tae. H. Rhew, and Sharon L. Melton, University of Tennessee, Agriculture-Food Tech, P.O. Box 1071, Knoxville, TN.

The triglyceride structure of naked seeded pumpkin seed oil (*Cucurbita pepo*, var. Lady Godiva) was determined by the 1, 3-random, 2-random theory and argentation thin-layer chromatography. Crude oil was extracted from dried, ground whole seeds that were alkali refined, bleached, and deodorized. The triglycerides of the refined oil were isolated and hydrolyzed by pancreatic lipase. The 2-monoglyceride was isolated from the hydrolyzate by thin-layer chromatography. Triglyceride fatty acid composition was determined to be 14.96% palmitic, 4.78% stearic, 49.11% oleic, 30.87% linoleic and 0.28% linolenic, and the fatty acid composition of the 2-monoglyceride was determined to be 3.56% palmitic, 0.98% stearic, 53.0% oleic, 42.11% linoleic, and 0.34% linolenic. The main triglyceride structures calculated by grouping fatty acids into saturated (S, palmitic and stearic), oleic (O), and unsaturated (U, linoleic and linolenic) were SOO-(13.58%), OOU (13.32%), OOO (12.38%), OUS (10.99%) and OUU (10.59%). Alignment structures of the triglyceride molecule where U = unsaturated fatty acid and S=saturated fatty acid were calculated to be 0.28% SSS, 1.63% SSU, 6.42% SUS, 2.32% USU, 36.75% SUU, and 52.58% UUU. Results obtained by the 1,3-random, 2-random theory will be compared with results obtained from argentation thin-layer chromatography.

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A STUDY OF THE FLAGELLAR LIPIDS OF *CHLAMYDOMONAS REINHARDII*. Paul C. Brown, City College of New York, Chemistry Department, New York, NY 10031.

Chlamydomonas reinhardii, a unicellular chlorophyte, mates by cell to cell contact at the tips of its flagella. In an attempt to examine glycolipids as the potent recognition factor, we have isolated the flagellar membrane and have studied its lipid composition. The lipid composition of the flagellar membrane is identical to that of whole flagella as detected by two dimensional thin-layer chromatography. The lipid extracts contain PC, PA, PE, PI, sterols and free fatty acids. The total lipids represent a remarkably small fraction of the total dry weight. There appear to be large polar glycolipids in the preparation. Studies on these substances and the source of the free fatty acids will be presented.

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PASSION FRUIT SEED AS A POTENTIAL OIL SOURCE. C.T. Sun, J.T. Lee and T.Y. Liu, Food Industry Research & Development Institute, P.O. Box 246, Hsinchu, Taiwan, 300, R.O.C.

The passion fruit (*Passiflora edulis*) seed which has been the refuse of juice production was studied for the possibility of being a new oil source. The proximate analyses of the dry seed, which was of 3.5% whole fruit, were 6% moisture, 25% oil, 15% crude protein (N x 5.30) and 57% crude fiber. The crude oil was obtained either by n-hexane extraction or hydraulic press and was analyzed for its properties including fatty acid composition. The result showed that the peroxide value of the crude oil was 2.3, acid value 0.5, iodine value 132, refractive index 1.4760 (20 C) and saponification value 191, linoleic acid about 60% and linolenic acid about 1%. The crude

oil was processed through caustic refining, bleaching and deodorization, and produced a refined oil with peroxide value below 1, acid value below 0.1, lovibond combination of 0.6 unit red and 1.9 units yellow in color and 14 hours AOM in stability. In view of the oil quality, the passion fruit seed might be a new oil source.

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CHARACTERISTICS OF NICKEL/SULFUR CATALYSTS IN THE HYDROGENATION OF SOYBEAN OIL AT *TRANS* ISOMER PRODUCING CONDITIONS. D.V. Okonek, W.R. Alcorn, and L.A. Cullo, Harshaw Chemical Company, 23800 Mercantile Road, P.O. Box 22126, Beachwood, OH 44122.

A family of catalysts with different Ni/S ratios was tested at 216 C and 5 psig for soybean oil hydrogenation. The trade-off between extent of hydrogenation and *trans* isomer yield is shown in a simple correlation. The sample with the highest sulfur content was then blended with unsulfided catalyst to a range of Ni/S ratios. Test results indicate that the optimum catalyst is obtained when prepared at a particular Ni/S ratio rather than through blending. In separate series of experiments, a commercial product (Harshaw Nysel SP-7) was tested at a variety of conditions. Results are given in terms of I.V.-time, *trans* isomer yield, product composition, solid fat index, and the effects of pressure, temperature, and catalyst concentration.

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HALOGEN POISONING OF Cu-BASED HYDROGENATION CATALYSTS. J.A. Heldal and P.C. Mørk, Laboratory of Industrial Chemistry, N-7034 Trondheim-Nth, Norway.

Marine oils may contain up to 100 ppm of halogens, mainly chlorine. To a lesser extent, halogen compounds may also be found in vegetable oils. The poisoning effect of such substances on Cu-based catalysts has been investigated. The deactivating effect of f.i. hexadecyl chloride was found to correspond to a loss of 0.004-0.006% Cu per ppm Cl added, increasing with decreasing amounts of Cl. The poisoning mechanism was found to involve a dehydrohalogenation of the alkyl chloride. The hexadecene formed was quantitatively determined by GLC analysis of the liquid phase. Most experiments were carried out on model systems with squalane as the continuous phase, because it was found that even well-refined and bleached soybean oils contained small amounts of unidentified catalyst poison.

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MODELING OF REACTION RATE CONSTANTS IN SOYBEAN OIL HYDROGENATION. A.H. Chen, D.D. McIntire, and R.R. Allen, Process Development, Anderson Clayton Foods, 3333 North Central Expressway, Richardson, TX 75080.

Reaction rate constants in soybean oil hydrogenation using Ni and Pd as catalysts have been modeled with semi-empirical models. The parameters were: temperature, agitation power per unit volume of oil, concentration of catalyst, and hydrogenation pressure. Arrhenius equation was used to describe the temperature function. Power laws were employed to relate the remaining parameters to the rate constants. The models gave satisfactory fit to the data. For both catalysts, increases in each parameter cause positive response to the rate constants.

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PARTIALLY HYDROGENATED-WINTERIZED SOYBEAN OIL. G.R. List, Northern Regional Research Center, AR, SEA, USDA, 1815 North University, Peoria, IL 61604.

Partially hydrogenated-winterized soybean oil has been a commercial reality since the early 1960s and represents a major advance in edible oil processing technology. Methods for preparing feed stocks for the winterization process will be reviewed, with emphasis on the hydrogenation parameters necessary to produce optimum yields of winterized oils. The winterization process will be reviewed along with methods used to separate the stearine from the liquid oil fraction. Such methods include horizontal pressure leaf and continuous vacuum filters. Composition and the flavor and oxidative stability of commercial and laboratory-produced products will be discussed. Equipment and utility cost estimates for a hydrogenation-winterization operation will be presented.

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KINETICS, MASS TRANSFER AND SCALE-UP IN NICKEL-CATALYZED OIL HYDROGENATORS. Alfred A. Susu, Ayodele F. Ogunye, and S. Ganeshalingam, Chemical Engineering Department, University of Lagos, Lagos, Nigeria.

The kinetics of the hydrogenation of refined groundnut oil was described by a Langmuir-Hinshelwood model. The activation energy of the rate-determining step was found to be 24 kcal/gmol, and the heat of adsorption for hydrogen on a commercial nickel catalyst was found to be 32 kcal/gmol. These constants (kinetic and thermodynamic) were then utilized in calculating the gas bubble mass transfer using a general expression relating mass transfer to chemical reaction. The calculated mass transfer coefficients were correlated with P/V, the power input by the impeller per unit volume. The following correlation was found good for the two reactor scales (0.31 and 3.81) used in this investigation:

$$K_bA_b = 0.027\ (P/V)^{0.34}\ min^{-1}$$

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OIL REFINERY DIGITAL CONTROL FOR CONTINUOUS CAUSTIC DILUTION. Peter F. Elliott, Elliott Automation Company, Inc., 7322 Hamilton Avenue, Cincinnati, OH 45231.

The reduction and control of oil refinery losses is of great concern to oil refiners everywhere. High edible oil prices and the increased cost of processing has made it more and more feasible to automate the refining process. Not surprisingly, the digital measurement of continuous refining loss was the first step into the age of electronics for many refiners. The next step, however, is just as necessary and involves critical and precise control of the process variables. In the continuous neutralization process, one of the most important variables is the caustic treat. Optimizing the refining process for minimum refining loss infers an ability to adjust the dry weight basis caustic treat as a percentage of the crude oil flow rate, and to provide control of the caustic dilution on a continuous dilution basis. A digital control system has been designed that will perform this task with great precision and reliability. This control system has worked very well ever since it was installed in a U.S.A. corn oil refinery about twelve months ago. The mechanical and electronic design concepts of this digital control system for continuous caustic dilution will be described in some detail. The need for this kind of precision in edible oil refineries will also be discussed and questions will be answered if time permits.

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COMPUTER APPLICATIONS TO EXTRACTION & OIL PROCESSING. N. Witte, Central Soya Co., Inc., 1300 Ft. Wayne Bank Bldg., Ft. Wayne, IN 46802.

There are potential applications for computers in extraction and oil processing plants. This paper covers an overview of some of the possibilities with some examples from the author's experience of the developmental problems and present applications.

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A COMPARISON OF ELECTROMECHANICAL AND MICROPROCESSOR BASED CONTROLS FOR THE FAT AND OIL INDUSTRY. G.E. Pearce and J. Coon, EMI Corporation, 3166 Des Plaines Avenue, Des Plaines, IL 60018.

Traditional control systems used in the fat and oil industry are reviewed, including: pneumatic analog loops, relays, timers, counters, and stepping-drum program controllers. The NEMA definition of a microprocessor-based programmable controller is presented along with the major components of such systems, including CPU, 1/0 modules, power supply, and programming devices. These are briefly discussed from a user viewpoint. The functional capability of programmable controllers to handle on/off signals, analog signals, and PID control loops are described. A presentation is developed concerning the major considerations that should be evaluated when comparing electromechanical and programmable controller systems for a specific service. In conclusion a pro and con comparison is made between the programmable controller and the older, conventional controls for typical fat and oil processes such as caustic refining, stock changing in deodorization, automated cyclic control of dual batch type filters in continuous processes, and the use of a programmable controller to replace mechanical time/process set point programmers for controlled application of vacuum to batch type reactors.

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BY-PRODUCT RECOVERY AND POLLUTION CONTROL BY FERMENTATION PROCESS IN OIL EXTRACTION PLANTS. Lars Malm, Department FTB, Alfa-Laval AB, Box 500, S-147 00, Tumba, Sweden.

In palm oil mills and similar mechanical extraction plants, the wasted liquid stream contains valuable, but hardly separable, remaining oil and solids. A new process for recovery of oil and ensilaged solids is presented. The new process has been tested on a full scale in a Malaysian palm oil mill. The liquid from the palm oil clarification station is mixed with a recycled flow from an enzyme generating tank and is then ensilaged in a fermentation reactor. The lactic acid produced maintains a low pH, and the temperature is controlled within the thermophilic range. The emulsifying properties of the process liquid change during the ensilage process, so that the oil can be separated from the liquid by a centrifugal separator. The ensilaged solids are removed and dewatered by a decanter centrifuge. The recovered oil has a low FFA content and other characteristics of a good quality palm oil. The ensilaged solids have potential as animal feed ingredients. Another advantage of the new recovery process is that the load of the waste water treatment plant is reduced to one half or less with a corresponding cost

reduction. Recycling of the recovery process effluent within the mill will reduce the costs for process water and waste water treatment additionally. Flow sheets, photos and operation data will be presented.

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INCORPORATION INTO LIPID CLASSES OF PRODUCTS FROM MICROSOMAL DESATURATION OF ISOMERIC *TRANS*-OCTADECENOIC ACIDS. T. Riisom and R.T. Holman, The Hormel Institute, 801 16th Avenue N.E., Austin, MN 55912.

Desaturation of labeled isomeric *trans* 18:1 acids by liver microsomes from essential fatty acid deficient rats has been shown to produce predominantly c,t-18:2 isomers (tΔ^6, tΔ^{13}), predominantly c,c-18:2 isomer (tΔ^5) or mixtures of c,t-and c,c-18:2 isomers (t-Δ^4, t-Δ^{11}, t-Δ^{12} and t-Δ^{14}). The objective of the present study was to determine the extent to which these desaturase products, which constitute unusual isomers of 18:2, are incorporated into lipid classes and in particular, to determine any lipid class preference or positional specificity in the incorporation. The labeled fatty acid composition of phospholipids (PL), triacylglycerols (TG), and cholesteryl esters (CE), and of the PL fractions of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were characterized and quantified following *trans* methylation and $AgNO_3$ TLC. The microsomal enzyme system, optimized for desaturation, incorporated t-18:1 substrates plus the c,c- and c, t-18:2 products into the microsomal lipids in the order CE>PL>TG. The same total amount of labeled compound was incorporated regardless of the fatty acid structure, but there were differences in the ratios of CE:PL:TG. The distribution of 18:2 isomers between PL, TG, CE, and the free fatty acid fraction (FA) showed preferences and varied with the structure of the acid.

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INFLUENCE OF A MARGARINE CONTAINING DIET ON ARYL HYDROCARBON HYDROXYLASE ACTIVITY. Joseph Sampugna, Mary G. Enig, and Mark Keeney, Department of Chemistry, University of Maryland, College Park, MD. 20742; James L. Casterline, Food & Drug Administration.

Aryl hydrocarbon hydroxylase (AHH) is one of the important enzyme systems involved in xenobiotic metabolism. The activity of this cytochrome P_1-450 (P-448) mediated monooxygenase(s) is influenced by various factors, including diet. As part of a project designed to assess the biochemical fate and effects of dietary *trans* fatty acids, we have examined the fatty acid composition and AHH activity in liver microsomes of mice (C57BL/6J, males) reared on a standard diet containing 10% fat derived from margarine (25% *trans*-octadecenoate). These data were compared to those obtained for mice raised on a standard diet containing 10% unhydrogenated vegetable fat (control diet) or a commercial laboratory chow diet. Compared to values obtained for mice raised on control or chow diets, specific activity of AHh was 2-3 times higher in liver microsomes isolated from mice fed the diet containing margarine fat. Based on evidence from glass capillary gas-liquid chromatography, *trans*-octadecenoate was present in microsomal lipid derived from mice raised on all three diets; however, the levels of *trans*-octadecenoate were 4-6 times higher in neutral and phospholipid fractions isolated from mice fed the diet containing margarine fat. Although the specific activity of AHH observed in this study appears to be related to the levels of *trans*-octadecenoate in the microsomal lipids, other factors in the margarine diet may be responsible for the elevated AHH activity.

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THE INFLUENCE OF PARTIALLY HYDROGENATED MARINE OILS ON LIPID METABOLISM IN LIVER AND HEART OF RATS. Gunhild Hølmer and Carl-Erik Høy, Department of Biochemistry and Nutrition, Technical University of Denmark, Bldg. 224, 2800 Lyngby, Denmark.

Partially hydrogenated marine oils (PHMO) containing high amounts of long chain monoenoic fatty acids (C 20 and C 22), and geometrical as well as positional isomers, have been shown to provoke lipidosis and probably long term lesions in the hearts of various animal species. In a feeding experiment with rats (16 weeks), it was found that PHMO greatly influenced the deposition of polyunsaturated fatty acids in rat liver mitochondrial membranes, thus changing the membrane integrity. Another feeding experiment was designed to test whether the long term lesions found in rat hearts after feeding them oils containing C 22 - monoenoic acids could be related to decreased contents of phospholipids and polyunsaturated fatty acids. The lipid composition of heart, liver, and depot fat will be presented, along with discussion as to whether the decrease is due to inhibition of the conversion mechanism for linoleic acid to arachidonic acid, or is the result of increased peroxisomal oxidation induced by long chain fatty acids.

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INFLUENCE OF PARTIALLY HYDROGENATED PEANUT OIL ON THE PHOSPHOLIPID COMPOSITION AND THE FUNCTION OF MITOCHONDRIA. Carl-Erik Høy and Gunhild Hølmer, Department of Biochemistry and Nutrition, Technical University of Denmark, Bldg. 224, 2800 Lyngby, Denmark.

The effect of dietary partially hydrogenated fats containing positional as well as geometrical isomeric fatty acids on the composition and function of the mitochondrial membrane was studied. Weanling male rats were fed diets containing partially was hydrogenated peanut oil (51.0% *trans* acids) with or without a supplement of linoleic acid for 16 or 26 weeks. At the end of the experimental periods, their liver and heart mitochondria were isolated. The respiratory function of the mitochondria was estimated from the activity of cytochrome oxidase (EC 1.9.3.1.). The phospholipid distribution and the fatty acid composition of the mitochondrial membranes were analyzed to reveal any structural changes of the membranes induced by the dietary fats.

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PARTIALLY HYDROGENATED MARINE OIL (PHMO), INFLUENCE ON RAT LIVER METABOLISM. Trond Farbu, Jan Andresen, Ragnhild Rønneberg,* and Georg Lambertsen, Government Vitamin Institute, Directorate of Fisheries, P.O. Box 4285, 5013 Nygardstangen, Bergen, Norway.

Partially hydrogenated marine oil (PHMO) was given to rats at a 20% level in several feeding experiments to compare results with those from feeding rats other dietary fats. Sunflower seed oil was added to the PHMO. Growth and digestion; liver triglyceride, protein and glycogen; plasma free fatty acids; and ketone bodies and glucose were among the factors studied. Lipid fatty acid compositions were determined by glass capillary GLC. Diets having marginal protein and choline contents resulted in increased liver triglyceride levels after feeding the rats fats other than PHMO. Persistently low liver triglyceride levels were seen in the PHMO-fed rats. Increases in liver protein and plasma ketone bodies, and a reduced level of plasma glucose in rats fed PHMO suggest a preferred –probably peroxisomal–catabolism of the PHMO fatty acids, rather than an incorporation into liver triglycerides.

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OCCURRENCE OF *TRANS* FATTY ACIDS IN NEWBORN MICE. Luke A. Pallansch, Lillian M. Tidler, Joseph Sampugna, and Mark Keeney, Department of Chemistry, University of Maryland, College Park, MD 20742.

Although it is believed that little if any *trans* fatty acids occur in newborn tissue, it has been reported that ilaidic acid is incorporated to the same extent as oleic acid in fetal tissue. Since our research centers on the fate and effects of dietary *trans* fatty acids, it is important to know whether these fatty acids are transferred from mother to fetus. Consequently, we have examined the uptake of maternal *trans* fatty acids into newborn mice. Two groups of C56BL/6J mice were raised on semi-synthetic diets that were identical in all respects except for fatty acid composition. One diet (*trans*) contained partially hydrogenated corn oil, while the other diet (control) was identical to the *trans* diet with the exception that *trans* fatty acid isomers were absent. Pregnant female mice, fed *ad libitum* from birth on these respective diets, were utilized as a source of newborn mice. At birth, pups were isolated before they had a chance to nurse, and total lipids were extracted employing the Folch procedure. Analysis of Folch lower phase lipids indicated that *trans*-octadecenoates were present in percentages up to 2.9% (by weight) in pups born to female mice raised on the *trans* diet. Folch upper phase lipids were separated into two fractions, one of which is believed to contain primarily gangliosides, as evidenced by thin-layer chromatography. *Trans*-octadecenoates were present in levels up to 3.4% in the ganglioside fraction, and the non-ganglioside upper-phase fraction contained even higher percentages of *trans* octadecenoic acid. Folch lower phase lipids of control tissue also contained a small percentage of a component with a relative retention time similar to *trans*-octadecenoate, as detected by gas capillary gas-liquid chromatography. Possible sources of this minor component will be discussed.

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FATTY ACID COMPOSITION OF SELECTED FOOD ITEMS WITH EMPHASIS ON *TRANS* OCTADECENOATE. Mary G. Enig, Luke A. Pallansch, Joseph Sampugna, and Mark Keeney, Department of Chemistry, University of Maryland, College Park, MD 20742.

Selected foods have been analyzed for fatty acid composition with emphasis on the *trans*-octadecenoate and *trans*-octadecedienoate content. Fatty acid methyl esters were separated using a glass capillary gas-liquid chromatographic column coated with SP 2340. *Trans*-octadecenoate ranges and *trans* octadecenoate + octadecedienoate ranges (latter in parentheses) as percent of total fatty acids were determined for more than 200 food items. Values were: bread and rolls, 1.8-23.6 (10.4-27.9); breadings, 8.1-32.7 (12.1-33.5); butter, < 0.1-1.2; cakes, 9.3-21.1 (10.1-24.0); candies, 0.1-4.1 (0.1-4.3); cookies, 11.4-34.2 (13.9-37.4); crackers, 2.2-

20.9 (2.8-31.6); french fries, 4.6-35.1 (4.6-37.4); frostings, 16.0-26.6 (16.7-27.3); instant and canned puddings, 28.4-35.1 (30.5-36.1); margarines/stick, 15.9-31.0 (18.0-36.0); margarines/soft, 10.2-17.6 (11.2-21.3); margarines/diet, 11.3-13.3 (13.7-17.9); mayonnaise and salad dressings, 2.8-3.3 (4.5-4.6); non-dairy creamers and toppings, 0.4-2.1; oils, 0.2-9.4 (0.4-13.4); pastries, pies and donuts, 1.4-32.1 (1.4-34.4); shortenings, 8.7-35.4 (13.0-37.3); snack chips and pretzels, 12.8-30.4 (14.4-33.4). Based upon these analyses, menus developed to comply with McGovern Committee Dietary Goals, Prudent Diet, or Alternative Diet were calculated to contain increased levels of *trans* fatty acids compared to current estimated intake levels. Since *trans* fatty acids resemble saturated fatty acids and have been reported to interfere with biological availability of essential fatty acids, we have calculated P/S ratios that include the *trans* fatty acids as "saturated" components. When this was done, all of the 38 margarines analyzed had a P/S value less than 2, and only one out of 22 stick and 10 out of 16 soft and diet margarines had a P/S value greater than 1.

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ANALYSIS OF LONG-CHAIN ISOMERIC FATTY ACIDS WITH HPLC AND GLASS CAPILLARY GAS CHROMATOGRAPHY. Lennart Svensson and Rolf Blomstrand, Dept. of Clinical Chemistry, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

Positional and geometrical isomers of monounsaturated long chain fatty acids have been analyzed by the combination of high performance liquid chromatography (HPLC) and glass capillary gas chromatography. A preparative group separation of *cis* and *trans* isomers of the monounsaturated fatty acid methyl esters was achieved according to the chain length by reverse phase chromatography, and using a highly sensitive refractive index detector. After collection of the different fractions containing *cis* and *trans* forms of the monounsaturated fatty acid methyl esters, the fractions were analyzed for the content of positional isomers using glass capillary gas chromatography. The described technique has been applied to the analysis of the isomeric monounsaturated fatty acid content in partially hydrogenated marine and vegetable oils, and of the degree of incorporation of different isomeric mononunsaturated fatty acids into membrane phospholipids of rat heart and liver mitochondria after feeding partially hydrogenated oils. The usefulness and the limitations of the technique are discussed.

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LIPID COMPOSITION AND METABOLISM OF THE PATAGONIAN BIVALVE MOLLUSC *DIPLODOM PATAGONICUS*. Ricardo J. Pollero and Rodolfo R. Brenner, Catedra de Bioquimica, Instituto de Fisiologia, Facultad de Ciencias Medicas, Universidad Nacional La Plata, Calle 60 y 120, 1900-La Plata, Argentina.

The composition of neutral lipids, phospholipids, and fatty acids, and their variations during the year was determined in the fresh-water mollusc *D. patagonicus*. The effect of temperature and starvation on the lipid composition and on transformations of fatty acids incorporated from the medium was also studied. Freshly collected animals from Lake Nahuel Huapi (Argentina) were used for seasonal variation studies. For the experimental work, animals kept in the laboratory at 9°C and 20°C for 8 weeks were used. The main lipids were choline and ethanolamine phosphatides, phosphonate aminoethyl ceramide, triacylglycerols, and sterols. Radioactive acids 18:2ω6 and 18:3ω3 were used as tracers to determine the metabolic fate of these fatty acids. The most important fatty acids were 16:0, 20:1ω9, 20:4ω6, and 18:2ω6. The high content of arachidonic acid (15 to 20%) and the low content of 20:5ω3 and 22:6ω3 acids (3% and 1% respectively) would indicate the strong influence of the freshwater milieu. Comparisons with the fatty acid compositions of estuarine species of Diplodom showed that the latter had values for these ω3 fatty acids intermediate between those of fresh-water *D. patagonicus* and marine bivalve molluscs. Starvation specifically decreased the content of triacylglycerols and glyceryl ethers. Modification of the temperature affected neither lipid nor fatty acid composition during the study period, but did change the mode of fatty acid incorporation.

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NUTRITIONAL ASPECTS OF MARINE LIPIDS IN CRUSTACEANS. Akio Kanazawa and Shin-ichi Teshima, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Japan.

At present, prawn culture techniques are being actively investigated in Japan and southeast Asian countries; however, little information is available on lipid nutritional requirements of prawns. Our experiments have demonstrated these requirements for larval prawns through feeding studies using artificial diets and radioisotopic analyses. Addition of cholesterol, linoleic, linolenic, eicosapentaenoic, or docosahexaenoic acids to the diet promoted growth in zoeal- and mysis-stage larvae of *Penaeus japonicus*. Nutritive values of eicosapentaenoic and docosahexaenoic acids were higher than those of linoleic and linolenic acids in *Penaeus* larvae. Metabolic tracer experiments showed that radio-labeled acetate is only slightly incorporated into cholesterol, linoleic, linolenic, and ω3 long-chained polyunsaturated fatty acids of *Penaeus* larvae. These results indicate that cholesterol, linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids, as previously shown for juvenile prawns, are also nutritionally essential for larvae.

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LIPIDS OF NERVOUS TISSUES FROM HORSESHOE CRAB AND HAGFISH. Richard Lee and Frances Gonsoulin, Skidaway Institute of Oceanography, P.O. Box 13687, Savannah, GA 31406.

The horseshoe crab, *Limulus polyphemus*, is an arthropod which evolved during the Cambrian age. The predominant lipids of its nonmyelinated nerves were cholesterol (11% of total lipid) and phospholipids (81%). The phospholipids were characterized by a high content of plasmalogens; i.e., alk-l-enyl-acyl-glycerophospholipids; 42% of the phosphatidylethanolamines was the plasmalogen, alk-l-enyl-acyl-phosphorylethanolamine. The fatty acids of the phospholipids, with the exception of spingomyelin, were high in polyunsaturation with 20:4 and 20:5 as the major fatty acids. Spingomyelin had predominantly long-chained saturated fatty acids. Cerebrosides and gangliosides were absent from horseshoe crab nerves. The hagfish, *Eptatretus deani*, belongs to the most primitive class of vertebrates, the Cyclostomata. Lipids of hagfish brain tissues included gangliosides (25%), phospholipids (35%), cerebrosides (8%), and cholesterol (12%).

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FATTY ACIDS ESSENTIAL FOR THE REARING OF FISH: INFLUENCE OF DIFFERENT YEASTS, *CHLORELLAS* AND ROTIFERS ON MORTALITY IN RED SEABREAM. Takeshi Watanabe, Laboratory of Fish Nutrition, Tokyo University of Fisheries, Konan 4-5-7, Minato-ku, Tokyo 108, Japan; Chikara Kitajima and Shiro Fujita, Nagasaki Pref. Inst. Fisheries; Osamu Imada, Kyowa Hakko Kogyo Co., Ltd.

Rotifers, *Brachionus plicatilis*, a living feed used extensively in the seed production of various kinds of fish in Japan, formerly was cultured with marine *Chlorella*. Baker's yeast, *Saccharomyces cerevisiae*, was then found to be more suitable as a culture medium. The rotifers cultured with yeast (Y-rotifers), however, resulted in sudden heavy losses of fish—one of the biggest problems in the rearing of fish in Japan, especially in the mass production of red seabream larvae. Recent studies have demonstrated that the nutritional quality of rotifers depends upon the content of fatty acids essential (EFA) for fish. Y-rotifers were quite low in their content of EFA, whereas the rotifers cultured with marine *Chlorella* (C-rotifers) were found to contain a high level of 20:5ω3, one of the marine fish EFA. The difference in the concentration of 20:5ω3 in the rotifers was found to correspond with the difference of fatty acid compositions between yeast and marine *Chlorella*. When Y-rotifers were cultured with marine *Chlorella*, the low content of 20:5ω3 in Y-rotifers increased in proportion to the culture period through the incorporation of 20:5ω3 from the marine *Chlorella*, reaching a maximum at around 28% after two days of feeding. Furthermore, the dietary value of Y-rotifers for red seabream larvae was found to be significantly improved by this secondary culture with marine *Chlorella* for more than six hours, but not by a culture with freshwater *Chlorella*. We have developed a new kind of yeast (ω-Yeast) as a culture medium for rotifers by adding fish oil or cuttlefish liver oil as a supplement to the culture medium of baker's yeast, resulting in ω-yeast producing rotifers with high contents of 20:5ω3 and 22:6ω3—the EFA for marine fish—and a high dietary value comparable to that of C-rotifers. Our results indicate that the high mortality observed frequently in red seabream larvae can be induced by feeding them Y-rotifers as their sole feed, and is due to an EFA deficiency in the fish.

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LIPID ANALYSIS OF A NATURAL SOUTH ATLANTIC FOOD CHAIN. Victor Jorge Moreno and Julia Elena Aizpun de Morenzo, Instituto Nacional de Investigacion y Desarrollo Pesquero, C.C. 175, Playa Grande, 7600 Mar del Plata, Argentina.

Natural populations of phytoplankton, zooplankton, anchovies *(Engraulis anchoita)* and hake *(Merluccius merluccius Hubbsi)*, trophically related, were analysed for their lipid and fatty acid composition. Collections of specimens were made from the same sea area near Mar del Plata, Buenos Aires Province, Argentina, at approximately monthly intervals. The fatty acid compositions of the four trophic levels were modified by ecological and physiological factors. Differences in species composition of phytoplankton communities apparently had no effect on the fatty acid pattern of their lipids. On the other hand, concentration ratios of 16:0/16:1 acids were more closely related to taxonomic differences. The fatty acid patterns of the zooplankton samples were similar but quantitatively different from those of phytoplankton. In zooplankton, compared with phytoplankton, the 20:5/22:6 acid ratio

was relatively lower and the 16:0/16:1 acid ratio was higher. Comparisons of the total fatty acid patterns from zooplankton and from anchovies corroborate the carnivorous habits of the latter. The different tissues of the hake showed a mean fatty acid composition that resembled that of the anchovies. Changes are shown to occur in both fishes depending on food availability and gonadal maturation, particularly in mesenteric, ovarian, hepatic, and muscle lipids, and in fatty acids.

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RELIABILITY OF FATTY ACID VALUES PURPORTING TO REPRESENT COMPOSITION OF OIL FROM DIFFERENT SPECIES OF FISH. Maurice E. Stansby, National Marine Fisheries Service, Northwest Fisheries Center, 2727 Montlake Blvd., E., Seattle, WA 98112.

More than 100 years ago, investigators began determining the proximate composition of many different species of fish. Because then it was not known that there was wide variation in fat content of individual fish of the same species, entirely inadequate sampling procedures were used, and, as a result, many values for fat content of species of fish were accepted into the literature and remained unquestioned for many decades. Today a similar situation is occurring for fatty acid composition of the oil of fish from different species. Starting in the early 1960s when GLC methodology became available, many investigations were undertaken from which the authors published results purporting to be representative for the fatty acid composition of fish of various fish species. In most cases quite inadequate samples were used, and this has resulted in publication of values usually representing only the fatty acid content of the few fish used in the investigation, which often bore no relationship to the fatty acid content for the species in general. Data will be presented to show the tremendous variation in fatty acid content of oils of fish of several species and discussion will be given on reasons for such variation, as well as how best in the future such problems can be resolved. Other possible sources of error, such as occasional failure to properly identify fatty acids, will be discussed briefly.

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LIPID CHANGES DURING EMBRYONIC DEVELOPMENT OF LAKE WHITEFISH, *COREGONUS CLUPEAFORMIS* (MITCHILL). M. Yurkowski, W.G. Franzin, H.R. Boese, and J.L. Tabachek, Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Canada R3T 2N6.

The lipid content and the composition of lipids and their fatty acids during different stages of embryogenesis of lake whitefish, *Coregonus clupeaformis* (Mitchill), were determined. The stages were unfertilized (UE), fertilized (FE), yolk sac encased (YEE), eyespot (ESE), otic cup (OCE), just before hatching (BHE) egg stages, and one-day old (F1) and 15-day old (F15) fry stages. The total lipid content (wet and dry weight basis) changed little throughout embryogenesis. The level (relative percent) of total neutral lipids (NL) (73 to 54%) decreased, and total phospholipids (PPL) (27 to 46%) increased from OCE to F15 stages. The level of triglycerides (TG) (90 to 73%) decreased, and that of sterol esters (SE) (4 to 6%), free sterols (FS) (6 to 18%), and diglycerides (DG) (0.2 to 1.4%) increased from OCE to F15 stages. The level of major PPL, phosphatidylcholine (PC) (80 to 61%), decreased from YEE to F15 stages, but that of phosphatidylethanolamine (PE) (8 to 16 to 10%) increased from YEE to F1 stages and then decreased after hatching. The level of minor PPL, phosphatidylserine (PS) (0.8 to 3%) and diphosphatidylglycerol (DPG) (1 to 8%) increased from OCE to F15 stages, and phosphatidic acid (PA) (1 to 3%) increased after the F1 stage. The level of phosphatidylinositol (PI), sphingomyelin (SPH), and lysophosphatidylcholine (LPC) changed very little. The major fatty acids (greater than 10% by weight) were: 16:0 (in all lipids); 16:1 (in TL, all NL, and LPC); 18:0 (in PI, PS, and PA); 18:1 (in all lipids except SPH); 20:4ω6 (in PI); 20:5ω3 (in TL, NL, SE, DG, TG, PPL, PC, PE, and LPC); 22:6ω3 (in all lipids except monoglycerides and SPH); 14:0 and 24:1 (in SPH). Embryogenesis had no effect on the fatty acid composition of TG, PC, LPC, and SPH, but altered the levels of some of the major fatty acids in the other lipids. In general, the levels of total saturated acids, 16:0, 18:0, total monoenoic acids, 16:1, 18:1, total ω6 acids, and 20:4ω6 decreased or remained constant and the levels of total ω3 acids, 20:5ω3, and 22:6ω3 increased or remained constant during embryogenesis.

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UTILIZATION OF MARINE OILS IN CHILE. Lilia Masson and María Angélica Mella, Facultad de Ciencias Químicas y Farmacologicas, Departamento de Química y Tecnología de Alimentos, Universidad de Chile, Casilla 233 Santiago Chile.

Chile has great potential for the production of marine oils because the country has approximately 4,000 km of coastline, not including its antarctic territory. Many species of fish are found along this coastline; some are utilized for direct human consumption whereas others are utilized for the production of fishmeal and oil. Among the latter are the anchovy *(Engraulis ringens)*, the sardine *(Sardinops sagax)*, the jurel *(Trachurus muphyi)*, and the merluza *(Merluccius gayi)*. Anchovy oil is highly unsaturated, averaging 10.0% eicosapentaenoic acid and 10.7% docosahexaenoic acid; its iodine value ranges from 170 to 184 and the principal fatty acid is palmitic acid (ave. 21.7%). In contrast, jurel oil is less unsaturated than anchovy oil; the iodine value ranges from 131 to 135 and the major fatty acid is eicosapentaenoic acid (ave. 13.8%). The production of fish oil in Chile was approximately 77,000 tons in 1978. A part of this was exported, but the remainder was retained and hydrogenated for use in the production of margarines and shortenings. The fatty acid composition of crude, fractionated, and refined fish oils, as well as that of some product margarines and shortenings, will be described.

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WHEAT FLOUR LIPIDS, SHORTENING, AND SURFACTANTS IN BREADMAKING: SYNERGISM AND/OR ANTAGONISM. O.K. Chung and Y. Pomeranz, U.S. Grain Marketing Research Laboratory, 1515 College Avenue, Manhattan, KS 66502.

Many variables govern the production of acceptable bread. They include wheat flour, dough formulations, bakeshop facilities, and the baker. This review covers the contribution of native wheat flour lipids, shortening, and surfactants to breadmaking. While all generally are beneficial when used alone, some combinations show synergistic, additive, or antagonistic effects. Shortening is essential in an optimum bread formula. For a positive shortening response, native flour lipids are required, and the degree of positive response depends on the amount and type of lipids present in flour. Small differences in amounts of free polar lipids in flours that vary in breadmaking quality accentuate differences in loaf volume potential of the flours: the better the inherent quality of flour, the greater the benefits derived from adding shortening. Surfactants can replace and/or displace flour lipids by interacting with starch components in slowing down staling, and by interacting with flour proteins or nonflour proteins in production of protein-enriched bread. Surfactants also can replace flour lipids and/or shortening. Optimum surfactant effects depend not only on the hydrophile-lipophile balance, the number of charged groups, and degree of polarity of surfactant itself, but also on the quantity and quality of lipids that are to be replaced or supplemented, the presence of shortening, and the quantity and nature of the protein-rich additives in the production of high protein breads.

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TRENDS IN THE USAGE OF SHORTENING IN BREADS AND ROLLS. Simon S. Jackel, Quality Bakers of America Coop., Inc., 1515 Broadway, New York, NY 10036.

The trend away from animal-based shortenings to vegetable products in the production of breads and rolls by the American baking industry has proceeded rapidly. It is estimated that more than 80% of wholesale bread production is made using vegetable shortenings. The major shortening by far is liquid soybean oil, which may or may not be lightly hydrogenated. Accompanying the switch from animal to vegetable shortening has been a trend to lower fat usage levels in wholesale bread and roll production. Whereas a 3% level of shortening based on flour was common only a few years ago, current usage levels are more nearly 2% percent based on flour. The switch to liquid vegetable oils, without sacrificing interior characteristics of the bakery foods, was facilitated by the availability of suitable food-grade dough conditioners to provide appropriate solid fat levels. These are now widely used by the wholesale baking industry at levels typically about .5% based on flour, Sodium Stearoyl-2-Lactylate, Polysorbate 60, Ethoxylated Monoglycerides and Succinylated Monoglycerides represent widely-used dough conditioners. The U.S. Food and Drug Administration recently removed the .5% ceiling on mono- and diglycerides, which may make it possible for shortening levels to be reduced still further. At this time it is not clear whether a trend to shortening levels appreciably below 2% based on flour will develop.

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SHELF-LIFE STUDIES OF HYDROGENATED VEGETABLE SHORTENINGS IN BAKED GOODS FOR MILITARY RATIONS. N.E. Harris, N. J. Kelley, B.L. Bell, D.E. Sherman, L. Cox, and D.E. Westcott, U.S. Army Natick Research & Development Command, Kansas Street, Natick, MA 01760.

This study was initiated to determine the stability of four "commercially available" 100+ hour AOM shortenings which have potential use in bakery items for military rations. Shortenings tested were soy/cottonseed with Tenox 4, soy/palm with Tenox 2, cottonseed/no antioxidant, and soybean with Tenox 4. Cottonseed was considered the control since this shortening and/or peanut-oil based fat are generally required in most military ration item specifications. The test shortenings were used in a dense ration, soda cracker, chocolate nut roll, pound cake, and orange nut roll (meal, combat,

individual formula) and orange nut roll (meal, ready-to-eat, individual formula). Long-term storage studies were run at both 21 and 38 C with taste panels and chemical assays at each withdrawal time period. The storage periods at 21 C were 0, 6, 9, 12, 18, 24, and 36 months, and at 38 C were 0, 1, 3, 6, 9, and 12 months. The crackers were stored in both air and vacuum packs in 603 x 700 cans. The cakes were packed in 200 x 300 cans. Panel results for crackers indicate that there is some advantage in vacuum packaging this product, especially if it is stored at 38 C. In general, the panel results indicated that the shortenings with soy/cottonseed blend were the poorest, even under vacuum conditions. Samples stored at 38 C were rated significantly poorer than their 21 C counterparts. In the canned baked goods such as the orange and chocolate nut rolls and the pound cake, there was progressive deterioration of the products on prolonged storage. Poundcake deteriorated more rapidly than the other products. In these products, those containing cottonseed shortening alone without antioxidant were significantly poorer, indicating a definite need for use of antioxidants.

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EVOLUTION OF SHORTENINGS IN BREAD. W. Michael Smith, Durkee Foods, 900 Union Commerce Bldg., Cleveland, OH 44115.

In bread products, the principal function of shortening is to impart tenderness and softness to the product by producing a lubricating action between the dough's gluten strands and starch granules. Until about a hundred years ago the principal shortening used by bakers was lard, mainly because, in contrast to other available fats such as tallow and marine oils, it possessed a pleasant flavor and a desirable plasticity. Whereas butter had the same attributes to an even greater degree, its high cost limited its use to the more expensive cake-type products. The introduction of the so-called "compound shortenings" in the late 1880s represented an attempt to utilize the more plentiful vegetable oils in a form that closely resembled the plastic properties of lard. Shortly after the turn of the century, the process of hydrogenation of oils was developed; this led to the manufacture of shortenings with functional characteristics that were superior to those of lard and the compound shortenings. In the 1930s another major advance in shortening technology occurred when emulsifiers in the form of mono- and diglycerides of fatty acids were introduced into shortenings. Up to this time, the plastic properties of a shortening were considered to be essential for its creaming and aerating function in cake production. Two significant consequences of further progress in shortening technology resulted from the introduction of emulsifiers: (1) it was realized that the major functional property of a shortening was actually determined by the nature and amount of its solid fat crystals rather than by its plastic character; and (2) the recognition that the plasticity of shortening does not play quite the essential role that was formerly attributed to it opened the way to the development of fluid shortenings with improved functions, increased economy, and greater convenience in handling. The first fluid shortening systems designed specifically for bread and other yeast-raised products were introduced in the early 1960s. The next major improvement in the fluid shortening concept came in the early 1970s when, in addition to mono- and diglycerides, a new emulsifier was incorporated. By 1977 a third generation of fluid shortening appeared on the market. With each modification, the functionality of the product improved, resulting in more efficient performance and yielding end products of greater and more uniform quality. In summary, fluid shortening systems continue to expand their application in bakeries for the production of yeast-raised products. They not only offer convenient scaling control, labor savings, and reduced ingredient costs, but more important, contribute on a consistent basis to the quality and uniformity of the baked bread products.

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FUNCTIONS AND REQUIREMENTS OF FATS AND EMULSIFIERS IN PREPARED CAKE MIXES. Kristie A. Painter, General Mills, Inc., 9000 Plymouth Ave. No., Minneapolis, MN 55427.

Accompanying demands for product changes in formulation, recipe preparation, and processing of prepared cake mixes, have been modifications in the shortening and emulsifier systems. The type and form of emulsifiers and fats used to meet these demands for product changes are presented. The effects of four emulsifier systems: mono-diglycerides, polyglycerol esters, propylene glycol monoesters, and glycerol lacto esters, on cake quality are reported. The importance of the crystalline structure of the base fat on cake quality is also discussed.

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AN IDEAL SURFACTANT IN BREADMAKING. Y. Pomeranz, U.S. Grain Marketing Research Laboratory, 1515 College Avenue, Manhattan, KS 66502.

Surfactants from wheat flour, oilseeds, or industrial sources are widely used in the production of bread acceptable to consumers. What make them "tick", and how and why are some more effective than others? This paper will review the requirements that should be met by an ideal surfactant in breadmaking and what is needed to meet those requirements. Those requirements will be discussed with regard to mixing stability, fermentation tolerance, loaf volume, crumb grain, and staling. The effectiveness of a surfactant will be considered in the context of the sum of the effects of all ingredients and their balance in various stages of breadmaking, as well as the interactions among wheat flour components and among wheat flour components and added ingredients. The use of several surfactants in the production of acceptable bread will be discussed. Results of baking bread containing relatively large amounts of crude fiber or oilseed proteins will be included.

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FATS AND OILS AND THEIR ROLE AS FUNCTIONAL INGREDIENTS IN THE BAKING INDUSTRY. Fred Eber, 215 Westport Road, Wilton, CT 06897.

Shortenings or any edible fats or oils are glyceryl esters of fatty acids. They can be defined as a mixture of mixed triglycerides, whether their origin is vegetable or animal sources. A triglyceride consists of three fatty acid molecules combined with one molecule of glycerol. The characteristic difference of fats and oils are thus related to the length of the fatty acid chains and their saturation. The first oil or fat products used by man undoubtedly were rendered from the carcasses of wild animals, and finally advanced to rendering the body fat from domestic animals. Lard, the body fat of hogs, became the most widely accepted fat used in the United States. Large strides have been made in fats and oils technology. The use of vegetable oils gradually supplanted lard as the major source of shortening. At first, compound shortenings were produced using liquid oils and portions of oleo stearine. The hydrogenation or hardening process marked was one of the major developments that enabled a manufacturer to produce a plastic solid shortening entirely from liquid oils. The next most important shortening development resulted from the utilization of emulsifiers (monodiglycerides) to produce a cake and icing shortening. This enabled the baker to develop a superior cake using a higher ratio of sugar to flour with additional moisture. The use of monodiglycerides and other sophisticated emulsifier systems paved the way for further development of many specialty plastic and fluid shortenings. In the fifties, another major development was the interesterfication or molecular rearrangement process. This process has been of great value in development of new superior lard based or animal and vegetable shortenings with improved crystal structured and performance advantages. The physical and chemical properties measured by melting point, plasticity, and crystalline form of fat are some of the factors in determining the suitability of a shortening or oil for a particular use in the baking industry. These technological developments by the shortening manufacturers have taken advantage of all available tools to produce a large variety of solid and fluid shortenings to satisfy the demands of the baking industry.

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POLYSILOXANE FLUIDS IN BAKING FATS–EFFECTS ON BAKERY PRODUCT CHARACTERISTICS. K. Lorenz, Department of Food Science and Nutrition, Colorado State University, Fort Collins, CO 80523.

Developments in silicone chemistry have made available to the manufacturers of baking and frying fats and oils a group of compounds which function as antifoaming agents, antioxidants, and compounds which raise the smoke point. The most useful type of silicone was found to be dimethyl polysiloxane. Siloxanes are available in a series of standard viscosities. They are clear, colorless, odorless, bland, thermally and chemically stable, shear stable, and soluble and emulsifiable with many organic materials. They affect bakery product characteristics, however, when used in baking and frying fats and oils. Dimethyl polysiloxanes affect the quality characteristics of white, yellow, and sponge cakes. Batter specific gravities increase and viscosities decrease with increasing amounts of the compounds. Cake volumes decrease and grains become more compact. Cookie spread factors decrease and the characteristic sugar cookie type top grain is lost because of dimethyl polysiloxane addition. Doughnuts and rosettes show higher fat absorption attributable to silicone fluids in fats and oils. The effect of these compounds is independent of the viscosity of the silicone fluids. The level of addition determines the extent of change in the quality of baked and fried products.

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HPLC OF PHOSPHOLIPIDS AND GLYCOLIPIDS. Firoze B. Jungalwala, E.K. Shriver Center, 200 Trapelo Road, Waltham, MA 02154.

HPLC technique has been employed for the analysis of phospholipids and glycolipids. Our approach involved utilization of highly sensitive U.V. detectors. Complex lipids absorb U.V. light in the region of 190–200 nm, due to the presence of one or more double bonds in the molecule. The response, however, is dependent upon

the degree of unsaturation and not upon the molar content. The method of choice for the quantitative analysis was derivatization to form highly UV absorbing stable products. Methods were developed for the quantitative perbenzoylation and HPLC analysis of cerebrosides, sulfatides, ceramides, and other neutral glycolipids. Reliable quantitation of these lipids up to around 50 picomole in a variety of samples of biological origin was achieved. The following phospholipids were derivatized and quantitated by HPLC: sphingomyelin, phosphatidylglycerol, ethanolamine and serine containing phosphoglycerides. Other amino group containing lipids such as sphingosine, psychosine, and sphingosyl phosphorylcholine were derivatized as their biphenylcarbonyl derivatives and quantitated. Reversed-phase HPLC separation of the molecular species of the following lipids was achieved on either μ-BondaPack-C_{18} or "Fatty Acid Analysis" column: sphingomyelins and phosphatidylcholines with detection at 203 nm; benzoylated sphingomyelins; benzoylated hydroxy- and nonhydroxy-fatty acid containing cerebrosides and sulfatides; and biphenylcarbonyl derivatives of various sphingosines. These methods were applied to the analysis of complex lipids in various biological materials.

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SEPARATION OF MOLECULAR SPECIES OF CERAMIDES AS BENZOYL AND *p*-NITROBENZOYL DERIVATIVES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. U.H. Do, P.T. Pei, S.L. McKinley and S. Ramachandran, Applied Science Division, Milton Roy Company Laboratory Group, P.O. Box 440, State College, PA 16801.

Molecular species of ceramides containing non-hydroxy fatty acids, after conversion to the corresponding benzoyl and *p*-nitrobenzoyl derivatives, were studied by high performance liquid chromatography (HPLC) using a silica column and reverse phase columns. Intact non-hydroxy ceramides derived from egg yolk sphingomyelins were separated into ten components by HPLC on a reverse phase column. Ceramides were separated on the reverse phase column primarily according to the number of carbon atoms and degree of unsaturation. Ceramides derived from short chain saturated fatty acids were eluted earlier than those from long chain saturated fatty acids. Ceramides derived from unsaturated fatty acids were eluted earlier than those derived from saturated fatty acids of the same number of carbon atoms. Interestingly, ceramides containing mono-unsaturated fatty acids eluted together with ceramides containing three-carbon less saturated fatty acids. N-palmitoyl-D-dihydrosphingosine were eluted immediately after N-palmitoyl-D-sphingosine and just before N-heptadecanoyl-D-sphingosine. Standard ceramides were prepared from D-sphingosine and free fatty acids by reactions involving reduction-oxidation and condensation. A typical preparation, N-heptadecanoyl-D-sphingosine, was characterized by infrared thin-layer chromatography and mass spectrometry. Data obtained by HPLC analysis will be compared with that obtained by gas-liquid chromatographic analysis.

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PURIFICATION OF NATURAL AND SYNTHETIC PHOSPHOLIPIDS USING RADIALLY COMPRESSED SILICA COLUMNS. Kanu M. Patel, CalBiochem-Behring Corp., 10933 North Torrey Pines Road, LaJolla, CA 92037, and James T. Sparrow, Baylor College of Medicine.

We have previously reported (J. Chromatogr. [1978] 150:542-547) the rapid separation of crude egg phospholipids using the Waters Prep LC/500 equipped with radially compressed silica columns eluted with chloroform, methanol, and water at a flow rate of 200 ml/min. The phosphatidylethanolamine was eluted with $CHCl_3$:MeOH:H_2O (60:30:2, v/v), and the phosphatidylcholine with $CHCl_3$:MeOH:H_2O (60:30:4). The column was flushed with $CHCl_3$:MeOH:H_2O (60:40:10) after each injection and re-equilibrated with the appropriate solvent mixture. The recovery of phospholipid was excellent. We report here the application of the same technique to the purification of other phospholipid mixtures. Crude soy lecithin was purified in the same manner as the egg phosphatidylcholine. The lipids from the delipidation of human serum high density lipoproteins were separated using $CHCl_3$:MeOH:H_2O (60:30:4) into neutral lipids, phosphatidylcholine, and sphingomyelin. We have also isolated phosphatidylserine and sphingomyelin from crude bovine brain phospholipids. We have recently reported (J. Lipid Res. [1979], 20:674-677), a convenient synthesis of phosphatidylcholines, starting from the cadmium chloride complex of glycerophosphorylcholine, fatty acid anhydride, and 4-pyrrolidinopyridine in dimethylsulfoxide:benzene (1:1) at 42° which resulted in high yields of products in 2–5 hours. The reaction mixture was diluted with $CHCl_3$:MeOH:H_2O (5:4:1) and passed through a mixed-bed ion exchange column, either Rexyn I-300 or IR45/IRC50, to remove $CdCl_2$ and 4-pyrrolidinopyridine; the solvent was evaporated and the residue injected onto the Prep LC/500 as described above. The fatty acid eluted first, then the DMSO followed by the phosphatidylcholine. The synthetic short-chain phosphatidylcholines eluted in 16–20 minutes; the unsaturated in ∿10 minutes, and the saturated long-chain in 12–14 minutes. The high capacity and short contact time of the lipid with silica make this technique fast, convenient, and economical for the purification of various phospholipids.

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DETERMINATION OF VITAMIN A, E, K AND UBIQUINONE. Kouichi Abe, Osamu Hiroshima, Satoru Ikenoya, Masahiko Ohmae and Kiyoshi Kawabe, Analytical Research Laboratories, Eisai Co., Ltd., 6-10, Koishikawa 4-chome Bunkyo-ku, Tokyo 112, Japan.

In an attempt to develop convenient methods for the determination of vitamins A, E, K, and ubiquinones in biological materials, we investigated high performance liquid chromatography (HPLC). Non-fibrous tissues such as serum or liver were extracted with n-hexane in the presence of alcohol, and fibrous tissues were extracted with n-hexane after saponification with alcoholic KOH. The extracts were applied directly to HPLC systems without pre-treatment such as column chromatography. The HPLC conditions for the separation of these compounds in the extracts were investigated using various normal or reverse phase columns. The best separation could be obtained under the conditions using Nucleosil C_{18} column and ethanol:H_2O for vitamin A and K, Permaphase ODS (or Nucleosil C_{18}) column and ethanol:H_2O for ubiquinones, LiChrosorb DIOL column and isopropyl ether:n-hexane for vitamin E. Furthermore, the simultaneous separation of vitamins A and E could be obtained with the use of Nucleosil NH_2 column, which made it easy to establish an on-column concentration system and eliminated solvent evaporation of the extract. Fluorescent vitamins A and E were detected by fluorometry directly, but non-fluorescent vitamin K was first derivatized to naphthoquinone form by reaction with ethanolic sodium borohydride, and then detected by fluorometry. The detection of ubiquinones was performed using a highly sensitive on-line spectrophotometer or an electrochemical detector. Our methods are simpler, more sensitive, and specific than other conventional methods and can be used as routine methods for the determination of these substances in biological materials such as serum (plasma), liver, heart, and brain.

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SEPARATION OF PRENYLQUINONES AND OTHER PRENYLLIPIDS BY ADSORPTION AND RESERVED-PHASE HPLC. H.K. Lichtenthaler and U. Prenzel, Botanical Institute, University of Karlsruhe, Kaiserstraße 12, D-7500 Karlsruhe 1, West Germany.

HPLC allows a quick separation of natural prenylquinones (ubiquinones, plastoquinones, tocoquinones) and prenylvitamins (vitamin E, vitamin K, provitamin A) from biological material by either adsorption or reversed-phase chromatography. The HPLC technique can also be applied for the separation of prenyl plant pigments (carotenoids, chlorophylls). The adsorption HPLC-System is comparable with the separation sequence found in adsorption TLC. The prenylquinones vitamin K_1 and plastoquinone-9 appear shortly after β-carotene (provitamin A) followed by α-tocopherol (vitamin E) and the ubiquinones. The more polar α-tocoquinone has the longest retention time. Prenyllipids with different length of side chains usually appear as one peak. Reversed-phase HPLC gives good separation of prenyl compounds with different lengths of the isoprenoid side chain. Vitamin K_1 or K_2homologues are resolvedas separate peaks, as well as the ubiquinones Q-6, Q-9 and Q-10. Prenyllipids with the same chain length but different number of double bonds in the chain appear as separate peaks: the geranylgeranyl-naphthoquinone (MK-4) before the phytyl-naphthoquinone (K_1-4), the free prenol geranylgeraniol before phytol and the tocotrienols before the corresponding tocopherols. Prenyllipids (tocopherols, naphthoquinones) that differ in the number or position of methyl groups in the aromatic nucleus are resolved as individual peaks.

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HPLC DETERMINATION OF GLYCOLIPIDS IN HARD RED WINTER WHEATS AND FLOURS THAT VARY IN BREADMAKING POTENTIAL. O.K. Chung and B.G. Howard, U.S. Grain Marketing Research Laboratory, 1515 College Avenue, Manhattan, KS 66502, and T.N. Tweeten and D.L. Wetzel, Kansas State University.

The major wheat glycolipids, digalactosyldiglycerides (DGDG) and monogalactosyldiglycerides (MGDG), were quantitated in polar fractions of free lipids extracted by petroleum ether from 21 samples of hard red winter (HRW) wheats or 23 samples of experimentally milled HRW flours. The flours varied widely in mixing time (7/8 to 9 min) and loaf volume (523 to 1053 cc/100 g flour). DGDG and MDDG were analyzed by HPLC using a 10 μ Spherisorb ODS column, methanol/water (90:10), and a highly sensitive refractive index detector with interferometric optics. Wheat and wheat flour free polar lipids contained 11.74 to 21.25% and 17.92 to 45.65% DGDG, and 4.23 to 13.81% and 12.31 to 26.29% MGDG, respectively. Amounts of DGDG ranged from 2.01 to 5.49 mg and 2.20 to 11.37 mg; MDGD ranged from 0.90 to 3.59 mg and

1.51 to 6.20 mg per 10 g (dry basis) wheat or flour, respectively. The linear correlation between DGDG of wheat and flour was significantly higher (0.889) than the correlation between MGDG of wheat and flour (0.647). There were significant linear correlations between loaf volume and DGDG (0.771 and 0.787), MGDG (0.532 and 0.745), and the sum of DGDG plus MGDG (0.723 and 0.804) for the wheats and flours, respectively. As the amount of DGDG was more significantly related to loaf volume potential of wheat than the amount of MGDG, monitoring the DGDG content in wheat or flour free lipids might be of potential use as an index of predicting loaf volume potential of HRW wheats and flours.

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EFFECTS OF COLUMN PACKINGS AND SOLVENT SYSTEMS ON THE SEPARATIONS OF GLYCOLIPIDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. Thomas N. Tweeten, Hewlett-Packard Co., Rt. 41 and Starr Road, Avondale, PA 19311; David L. Wetzel, Kansas State University and O.K. Chung, USDA-SEA.

The high performance liquid chromatographic conditions have been studied for quantitatively determining digalactosyl diglyceride and monogalactosyl diglyceride from the petroleumether extract of wheat flour. Specifically, we have examined the effects of mobile phase strength, column packing stationary phase, packing particle size, and column temperature on both the efficiency and selectivity of the glycolipid separation. Glycolipids were detected using the variable wavelength UV detector at 200 nm. A comparison will be made of results obtained on LiChrosorb RP-8, LiChrosorb RP-18, and Spherisorb ODS columns. Observations of the effect of column temperature on the efficiency of separation will be reported.

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THE ANALYSIS OF DETERGENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. I. Chappell.

Not available at press time.

209

A UNIQUE NEW COSMETIC FLUID EMOLLIENT. Herman Brown, Finetex, Inc., 418 Falmouth Avenue, Elmwood Park, NJ 07407.

A new, commercially available patent-pending ester has the following unique properties: 1. a dry, lubricating feel to the skin (nongreasy). 2. a dry, lubricating feel to the skin even in the presence of large amounts of mineral oil; 3. safe and nonirritating quality; 4. solubility in most organic solvents; 5. acts as solubilizing agent for perfume oils and perfume agents; 6. acts as suspending agent assistant for aluminum chlorohydrin (in antiperspirants); 7. readily emulsifiable quality; 8. a low freezing point and high boiling point; 9. practically no odor or color. These properties plus others lend themselves to formulating this ester in a variety of cosmetic and toiletry products possessing a dry (nongreasy) lubricating feel. Examples of such cosmetic and toiletry products are skin creams and lotions, electric preshave lotions, perfume and colognes, body oils, bath oils, bath and shower gels, sunscreen and suntan oils and lotions, eye and face make-ups, shave creams and gels, deodorants and antiperspirants (all forms), nail polish removers and conditioners, hair grooms, hair conditioners, and shampoos. Noncosmetic products such as insect repellents can be prepared with the feel of a cosmetic product. Ointment bases as vehicles for drugs can be formulated to impart a dry emollient feel.

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JOJOBA OIL AS COSMETIC BASE. Thomas K. Miwa, Jojoba Plantation Products, Inc., 505 South Rockford Drive, Tempe, AZ 85281.

The cosmetically nontoxic nature of jojoba oil was not documented with experimental data until 1975, even though the oil has been used by American Indians and Mexicans for many centuries. Similarly, the chemical composition was not accurately determined until a decade ago, even though the liquid wax nature has been known for almost half a century. Formulation of jojoba oil into cosmetics and its quality control have been facilitated by knowledge of the constitution of the oil. Jojoba oil differs in many respects from its closest chemical analog, sperm whale oil. It contains no triglycerides, and its liquid wax ester average molecular size is 8 methylene units greater than the average sperm oil liquid wax ester.

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THE USE OF SODIUM BOROHYDRIDE IN PURIFICATION OF LANOLIN, TALLOW, AND OTHER RELATED DERIVATIVES. Michael M. Cook, Thiokol Corp./Ventron Division, Congress Street, Beverly, MA 01915.

This presentation will review the use of sodium borohydride for purification of a variety of natural oils such as lanolin, tallow, mink oil, and their related products. Sodium borohydride (SBH) provides a means of chemically reducing low-level impurities such as aldehydes, ketones, and metal complexes, which cause odor or color in these products or their subsequent derivatives. The results of adding sodium borohydride at various points during processing and/or conversion of these materials will be compared. For example, in the purification of tallow, following treatment with bleaching clay, the addition of 250–500 mg of sodium borohydride/kg tallow gives a significant reduction, primarily in the Lovibond red color. SBH reduction of the aldehydic impurities in the lanolin alcohols can prevent color formation in subsequent ethoxylated or sulfated derivatives. In addition, sodium borohydride has also been found to decolor a variety of natural oils such as mink oil during processing. Sodium borohydride offers a safe, effective method of improving product quality with only minimal changes in existing processing methods.

212

NEWER PROPOXYLATED DERIVATIVES AS RAW MATERIALS FOR COSMETICS. Graham Barker and Martin Barabash, Witco Chemical Corp., Technical Center, P.O. Box 110, Oakland, NJ 07436.

The propoxylation of quaternaries, fatty alcohols, fatty acids, and lanolin derivatives results in products that are becoming increasingly important as raw materials for cosmetic formulations. The high degree of branching and polarity imparted by the polypropylene glycol moiety results in highly desirable characteristics such as emollience, lubricity, solvation, improved spreading, low temperature stability, and dispersants for nonaqueous systems. Utilization of these derivatives in a number of cosmetic formulations will be demonstrated.

213

CONTROLLING N-NITROSO COMPOUNDS IN COSMETICS. William J. Mergens, Hoffmann-La Roche Inc., Nutley, NJ 07110.

The use of amines or amides as ingredients in cosmetic formulations, and the presence of nitrite as a contaminant or the presence of components containing nitro groups can lead to the formation of nitrosamines and nitrosamides, many of which are known carcinogens. Mechanism of reactions leading to the formation of nitrosamines and nitrosamides will be discussed. These can vary considerably, depending upon the nature of the particular formulation. Means of reducing or effectively minimizing these nitrosation reactions include removal of sources of nitrosating agents, avoiding undue exposure to air, and the addition of blocking agents such as ascorbic acid, ascorbyl palmitate, or tocopherol, which operate by reduction of the nitrosating agent. Thus, these substances compete with the amine or amide in these reactions.

214

ALPHA OLEFIN SULFONATES–COSMETIC INGREDIENT FOR THE 80s. A.M. Wendell, Shell Chemical Co., P.O. Box 2463, One Shell Plaza, Houston, TX 77001.

The manufacturing and applications technology for alpha olefin sulfonates (AOS) has undergone considerable development since the products first became available on a commercial scale during the late 1960s. AOS is no longer a curiosity, and should be considered an attractive candidate ingredient for shampoos, bubble baths and bar soaps, where mild, high foaming surfactants are required. This paper reviews the chemistry and manufacture of alpha olefins and AOS, compares AOS with other anionic surfactants, and discusses the key attributes that make AOS of interest to the toiletries industry.

214A

USE OF SILICONES IN COSMETICS AND TOILETRIES. Samuel R. Wendel, C40103, Dow Corning Corporation, 2200 W. Salzburg Road, Midland, MI 48640.

The first commercial appearance of a silicone in a cosmetic or toiletry application occurred in 1950, shortly after the inception of the commercial silicone industry in the mid-1940s. As with other ingredients in personal care products, silicones are used because of specific properties they convey to these formulations. Silicones find wide application in cosmetics and toiletries due to the facile variation of the silicone macromolecule. Specificially, silicone variability is manifested in terms of molecular weight variation and incorporation of diverse organic and silicone functionality. These variations affect both the physical and chemical properties of the silicone molecule. The uses of silicones in cosmetics and toiletries will be reviewed according to the physical and chemical properties of various types of silicones, and the performance parameters of personal care formulations affected by incorporation of specific silicone types.

215.

THE INFLUENCE OF OIL CARRIER LECITHINS ON METAL OXIDES AND THEIR DISPERSIONS. Max Kronstein, Manhattan College, Chemistry Department, Manhattan College Parkway, Riverdale, New York, NY 10471.

Oil carrier lecithins are the natural form in which vegetable lecithins are obtained from the various plant seeds. They have

specific properties owing to their lecithin groupings, and others owing to their oil carrier fraction. These properties make them highly useful materials for industrial applications. Both fractions can be separated by treating the oil carrier lecithins with selected solvents, in particular acetone, whereby the oily fraction is miscible with the acetone solution and the phosphatide fraction is acetone-insoluble. The isolated oil carrier fraction which is obtained from the acetone solution is still a different material from the glycerin fatty acid-ester oil which is obtained from the same seed. Some of these properties have been investigated and are discussed. The paper studies in particular the influence which the oil carrier lecithins and their isolated oil carrier fractions have in use with metal oxides, where they not only serve as wetting agents but exhibit other effects also. When metal oxides are used as pigments in various coating materials, and these lecithins (or their oil carrier fraction) are introduced into the prepared coating compound, it will result in a stable dispersion of the pigmentation in the vehicle and a ready redispersion of high specific gravity pigment materials which might have settled out upon extended storage of the paints. Pigments which have first been treated with these lecithins show a ready dispersion, and redispersion after storage, in the coating compounds. Examples are given of lecithin-metal oxide compositions and of their use in paints and in film-forming materials.

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LECITHIN IN MODERN COSMETICS. G.S. Kass, G.S. Kass and Associates, Ltd., 8938 N. Keeler Ave., Skokie, IL 60076.

Lecithin has never been used in cosmetics to any significant extent because of its formerly objectionable odor, color, and limited solubility characteristics. With the commercial availability of high purity lecithin, plus new techniques for solubilizing lecithin in aqueous cosmetic systems, there is renewed interest in lecithin as a cosmetic ingredient. This presentation will deal with its potential benefits in cosmetics and describe techniques for incorporating lecithin into various cosmetic systems.

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THE USE OF LECITHIN IN CALF MILK REPLACERS. A.B.M. Cloosterman, Unimills GmbH, Dammtorwall 15, D-2000 Hamburg 36, Federal Republic of Germany.

In the production of calf milk replacers (CMR) two basic processes can be applied–the spray drying method, and the direct fat addition method. In the spray drying method, the casein plays a very important role in preventing the coalescence of the fat droplets and promoting the formation of a stable emulsion. For reasons connected with the nature of the process, these two requirements are not fulfilled in the direct fat addition method, despite the fact that casein is generally present. A separate emulsion/stabilizer system must therefore be incorporated when applying the direct fat addition method. One good and widely applied emulsifier/stabilizer system is the combination of a synthetic emulsifier with a natural lecithin. An even better alternative is the use of a self-emulsifying lecithin, especially an enzymatically hydrolyzed lecithin. An additional and important effect of the use of lecithin in CMRs is that digestibility of the fat is markedly increased. It is for this reason that the incorporation of the lecithin into spray-dried products is highly recommended.

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CONTROL OF POSTHARVEST GLYCOALKALOID AND CHLOROPHYLL FORMATION IN POTATO TUBERS BY TREATMENTS WITH LECITHIN, OILS, AND HYDROCARBON COMPOUNDS. Karl B. Adams, Central Soya Co., Inc., and D.K. Salunkhe, Utah State University, UMC 87, Logan, UT 84322.

Light and mechanical injury are the two most important environmental stimuli for the postharvest synthesis of glycoalkaloids and chlorophylls. Partial or complete inhibition of light-induced chlorophyll and glycoalkaloid formation has been achieved by coating potato cultivar with various lipid or hydrocarbon based compounds. Applying hot wax to potatoes was shown to completely inhibit chlorophyll and solanine formation. Waxing was also effective for controlling sprouting. Coating potato cultivars with various vegetable oils or mineral oil at 22 C significantly inhibited the synthesis of chlorophyll and solanine. Complete inhibition of chlorophyll and solanine synthesis was accomplished by applying oils at higher temperatures. Spray treatments of potato tubers with commercial lecithin sprays significantly inhibited light-induced greening and glycoalkaloid formation. Lecithin-acetone solutions, and hydroxylated lecithin-acetone solutions, applied to potato tubers as dips, inhibited chlorophyll and glycoalkaloid synthesis. As the lecithin or hydroxylated lecithin concentration increased from 5 to 20%, the inhibition of glycoalkaloid increased, with 20% solutions resulting in complete inhibition of glycoalkaloid synthesis.

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THE FUNCTION OF PHOSPHOLIPIDS OF SOYBEAN LECITHIN IN EMULSIONS. Lisbeth Rydhag and Inga Wilton, The Swedish Institute for Surface Chemistry, Box 5607, S-114 86, Stockholm, Sweden.

A number of commercially available soybean lecithins were analyzed with respect to their phospholipid composition and emulsifying properties. It is well known that a phosphatidyl choline (PC) from soybean swells to a lamellar liquid crystalline phase that incorporates slightly less than 50% water. The swelling behavior of the commercially available soybean lecithins may be different, depending on the concentration of other phospholipids such as phosphatidyl ethanolamin (PE), phosphatidyl inositol (PI), and phosphatidic acid (PA). In the presence of the negatively-charged phospholipids PI and PA the swelling of the lamellar phase of PC was dramatically enhanced; however, a lecithin with equal amounts of PC and PE and small quantities of PI and PA formed two liquid crystalline phases–a lamellar and a hexagonal phase. It had earlier been shown that the presence of such structures may improve the emulsion stability. The function of the different soybean lecithins as emulsifiers is therefore going to be discussed with respect to thier swelling properties in water-oil systems.

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SOME PHYSICO-CHEMICAL PROPERTIES OF SOYBEAN LECITHINS IN RELATIONSHIP TO THE PHOSPHOLIPID COMPOSITION. Inga Wilton, The Swedish Institute for Surface Chemistry, Box 5607, S-114 86 Stockholm, Sweden.

When water is added to soybean lecithins, the phospholipids and the other surface active lipids will swell, forming phases with liquid crystalline structure. It is known that multilayers of such phases oriented at the interface between oil and water can enhance the emulsion stability. Using x-ray analyses of different samples of soybean lecithins that has been swollen with water, two liquid crystalline modifications have been found. The phospholipid compositions of the different samples were simultaneously analyzed and a relationship between the composition and the phase modifications was hypothesized.

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THE SURFACE CHEMISTRY OF LECITHIN: EFFECT OF UNSATURATION ON MOLECULAR INTERACTIONS. D.O. Shah, Department of Chemical Engineering, University of Florida, Gainesville, FL 32611.

An understanding of the surface chemistry of lecithin is very important for its use in biomedical and engineering systems and processes. Lecithin is a zwitterionic molecule consisting of a negatively charged phosphate group and a positively charged quaternary ammonium group. Thus, lecithin molecules can interact with cations, anions or can remain neutral, depending upon physicochemical conditions such as pH, the presence of di- or trivalent cations, etc. Using a monolayer approach, it was shown that the molecular area of lecithin increased as the degree of unsaturation increased. For four lecithins studied, the molecular area increased in the following order: Dipalmitoyl lecithin<egg lecithin<soybean lecithin<Dioleoyl lecithin. The calculated intermolecular spacing in monolayer increased by a fraction of an Angstrom. Such a small change in intermolecular spacing between lecithin molecules has a striking effect on the binding of di- or trivalent cations to lecithin molecules, and their enzymic hydrolysis behavior. The degree of unsaturation of fatty acid chains also influences the ionic structure of lecithin molecules, and packing characteristics with cholesterol molecules. In summary, the degree of unsaturation of fatty acid chains influences the area per molecule, intermolecular distance, binding of cations, enzymic susceptibility, and fluidity of lecithin monolayers.

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BIOCHEMICAL EFFECTS OF 4-HYDROXYALKENALS, IN PARTICULAR 4-HYDROXYNONENAL PRODUCED BY MICROSOMAL LIPID PEROXIDATION. Hermann Esterbauer, Institut für Biochemie, Universität Graz, Halbärthgasse 5, A-8010 Graz, Austria, and Mario Comporti and Angelo Benedetti, Institute of General Pathology, University of Siena, Italy.

Peroxidation of unsaturated lipids in biological membranes is believed to play an important role in many conditions of cellular damage, including the liver injury induced by some haloalkanes. Our studies indicate that cytotoxic aldehydes, in particular 4-hydroxynonenal, originating from the peroxidative breakdown of membrane lipids are, at least in part, responsible for the pathological consequences of lipid peroxidation. It has been shown that during the peroxidation of liver microsomal lipids, products are formed which have the capacity of inducing cytopathological effects (inhibition of microsomal glucose-6-phosphatase and cytochrome P_{450}, lysis of erythrocytes, and others). The products are dialysable and can be recovered in extracts obtained from the dialysate. After separation of the products present in the dialysate extract by TLC, the highest toxicological activity has been found in a band which contains most of the carbonyl functions present in the unfractionated extract. The products present in this TLC band have been identified as 4-hy-

droxyalkenals (almost entirely 4-hydroxynonenal [HNE]) by means of UV, IR, MS and HPLC of the free compound and its 2,4-dinitrophenylhydrazone. Synthetic HNE has been found to reproduce the cytopathological effects brought about by biogenic HNE. Hydroxyalkenals exhibit a high reactivity towards thiols (glutathione) and SH groups in proteins and enzymes, and it seems likely that the interaction with SH groups of biological importance leads to the impairment of many cellular functions. Hydroxyalkenals inhibit the synthesis of DNA, RNA, and protein, and glycolysis and respiration in tumor cells. They block mitosis of synchronized cultures of human kidney T cells and reduce the mitotic index of bone marrow when injected into rats. In cell free systems these aldehydes inhibit mitochondrial O_2 uptake and phosphate transport, protein synthesis, and functions of microtubular systems. The ID_{50} (aldehyde concentration giving 50% inhibition) for the various biological effects ranged between 0.05 and 0.5 mM.

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LEVELS OF OXYGENATED FATTY ACIDS IN YOUNG PLANT SEEDLINGS. Brady A. Vick and Don C. Zimmerman, Department of Biochemistry, North Dakota State University, Fargo, ND 58105.

13-Hydroperoxylinolenic acid, which results from lipoxygenase catalysis, can be metabolized by several plant enzymes. Of these, hydroperoxide isomerase converts this compound to either a 12,13-ketol (18:2) or a 9,12-ketol (18:2). Hydroperoxide cyclase converts 13-hydroperoxylinolenic acid to 12-oxo-phytodienoic acid (12-oxo-PDA), which is a cyclic fatty acid containing a cyclopentenone ring. The levels of 12,13-ketol (18:2) and 12-oxo-PDA were measured in the etiolated seedlings of 6-day-old sunflowers. The seedlings were extracted with hexane-isopropanol (3:2) containing 14,15-ketol (20:2) and the 20 carbon analog of 12-oxo-PDA as internal standards. A thin-layer chromatography fraction containing the compounds was esterified, and the methyloxime and trimethylsilyl derivatives were prepared. Selected ion monitoring by GC-MS showed that the level of 12,13-ketol (18:2) was 955 ng per gram fresh weight, or 655 ng per seedling. For 12-oxo-PDA the amount was 75 ng per gram fresh weight, or 51 ng per seedling.

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RELATIONSHIP BETWEEN LIPOPEROXIDE AND GERIATRIC DISEASES. Kazuo Fukuzumi, 11 Nunoike-cho, Higashi-ku, Nagoya, Japan.

The existence of *trans,trans*-conjugated diene hydroperoxides (a kind of lipoperoxides) in atherosclerotic arteries and cancerous tissues was first demonstrated and the research published in 1961 and 1963 by this author and others. Later, in 1965 and then 1969, this author proposed the lipoperoxide theory to explain many phenomena concerning atherosclerosis and cancer. The theory that cancer itself might be DNA radicals was presented in international congresses in 1972 and 1974 by the author, then published in an article in 1978. The Japanese suffer more from cerebral apoplexy and less from heart disease than Americans or Europeans. The reason for these phenomena has yet to be clarified; however, the lipoperoxide theory does offer some insight into the causes. The more the unsaturation the lipid, the more hydrogen bonds between the $-CH=CH-$ group and the $>NH$, the $-C\overset{O}{\underset{OH}{<}}$ groups in protein, or the $-OH$ group in water. Consequently, the lipid becomes covered with water-soluble protein, and the affinity between the lipid and water become stronger. The phenomenon that carcinogenic substances have the anticancer effect is named Haddow's paradox. The above-mentioned theories do not explain this phenomenon. If cancer is generated with lipoperoxide on the basis of lipoperoxide theory, the radical initiation reaction is connected with cancer generation. If cancer itself might be DNA radicals, the radical termination reaction relates to anticancer. Accordingly, carcinogenic substances connected with radicals naturally have the anticancer effect, and thus Haddow's paradox can be resolved.

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RATES AND PRODUCTS IN AUTOXIDATION OF PHOSPHATIDYLCHOLINE LIPOSOMES. Guey-Shuang Wu, Robert A. Stein, and James F. Mead, Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, Los Angeles, CA 90024.

Small phosphatidylcholine (PC) vesicles have been chosen as a model to study autoxidative processes in biomembranes. The autoxidation of pure soybean PC (containing 12% 18:1, 70% 18:2, and 6% 18:3 as major unsaturated fatty acids) at 40° was found to give mainly hydroperoxides, consisting of 45% 13-hydroperoxy and 33% 9-hydroperoxy fatty acids in the total product isolated. The disappearance of both 18:2 and 18:3 follows the classical autocatalytic type kinetics. When a large quantity of dipalmitoyl PC was incorporated into soybean PC liposomes, the rate of autoxidation was depressed considerably and both kinetics and product pattern were changed from those of pure soybean PC with the comparable extent of conversion. The major products include epoxy, hydroxy-epoxy, dihydroxy and trihydroxy fatty acids in the proportions of 5%, 13%, 10% and 45%, respectively. The production of hydroperoxide was found to be very much suppressed, with 13-hydroperoxide being only 10%, and 9-hydroperoxide only 16% of the total product. The structures of all the products were unequivocally established by GC/MS. Previously we also observed a similar effect in autoxidation of linoleic acid monolayers with added saturated fatty acid. Hypotheses will be advanced to rationalize these findings.

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FORMATION OF 13-L-HYDROPEROXIDE FROM LINOLENIC ACID IN TEA CHLOROPLASTS. Akikazu Hatanaka, Tadahiko Kajiwara, and Jiro Sekiya, Department of Agricultural Chemistry, Yamaguchi University, Yamaguchi 753, Japan.

Endogenous linolenic acid in *Thea* chloroplasts was cleaved into *cis*-3-hexenal and 11-formyl-*cis*-undecenoic acid via a very labile intermediate by E_2 of an enzyme system E_2 (E_2 and $E_2' + E_2''$) bound to the lamellae membranes of chloroplasts under aerobic condition. A large amount of linoleic acid was added to chloroplasts, and E_2' and E_2'' activities newly were induced in addition to E_2 activity. E_2' catalyzed the formation of 13-Hydroperoxide, then was cleaved to *n*-hexanal by E_2''. The enantiomeric composition of the 13-hydroperoxide produced by E_2' was determined by GLC and NMR analysis. After a large amount of linoleic acid was incubated with tea chloroplasts, a mixture of hydroperoxides (13-hydroperoxy-*cis*-9,*trans*-11-/9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid=84/16:crude-I) was isolated. The major hydroperoxide of the crude-I was identified as 13-L-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (80) containing a small amount of its enantiomer (13-D=20). So, it was demonstrated that E_2' in tea chloroplasts catalyzes the stereospecific oxygenation of linoleic acid to the 13-L-hydroperoxide.

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ESTIMATION OF VITAMIN E RELATED FLUORESCENT COMPOUNDS IN TISSUES. A. Saari Csallany and John D. Manwaring, University of Minnesota, Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108.

Chromatographic separation and quantitation of vitamin E-related fluorescent compounds from tissues were performed. Two groups of ten female weanling mice were fed for 14 months a basal diet containing 8% distilled corn oil. The basal diet was adequate in all respects except for vitamin E. One group received the basal diet, and the other group was supplemented with a high level of vitamin E (300 ppm d-α-tocopherol acetate). The lung, liver, spleen, kidney, heart, and brain tissues were homogenized and extracted with 2:1 chloroform:methanol. The organic phase was dried and water phase was lyophilized. The fluorescent compounds were separated in each phase by Sephadex LH-20 and G-25 column chromatography, respectively. For each tissue, thorough excitation and emission spectra were determined for the eluting fractions, then the fluorescence of each fraction was measured at the resulting excitation and emission maxima. Up to five fluorescent water-soluble compounds and one fluorescent organic solvent-soluble compound responded significantly to dietary vitamin E, depending on the tissue. Vitamin E in the diet led to the decrease of the organic solvent-soluble compound and four of the water-soluble fluorescent compounds. Respectively, their excitation and emission maxima were: 350 nm/435 nm/275 nm/350 nm, 270 nm/310 nm, 445 nm/520 nm, and 275 nm/350 nm. The fifth water-soluble fluorescent compound, which had excitation and emission maxima at 320 nm/380 nm, respectively, significantly increased these maxima due to 300 ppm vitamin E in the diet. This possibly indicates a protection of this fluorescent compound by vitamin E. The identification of these vitamin E-related compounds are being investigated.

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THE ROLE OF GLUTATHIONE PEROXIDASE IN P-450-MEDIATED DRUG-INDUCED LIPID PEROXIDATION. Albrecht Wendel and Sylvia Feuerstein, Physiologisch-chemisches Institut der Universität, Hoppe-Seyler-Str.1, D-7400 Tübingen, West Germany.

The hydrocarbon exhalation of starved mice was used as a tool for in vivo measuring of drug-induced lipid peroxidation. Acute paracetamol intoxication of these animals led to a dose-dependent lipid peroxidation (150 nmoles ethane/kg hr at 500 mg/kg), while the hepatic glutathione level fell to 10-20%. Pretreatment of the mice with the cytochrome P-450 inducer phenobarbital caused an increase of lipid peroxidation by factor of 10; treatment with the P-448 inducers caused increases of benzo (α)pyrene or methylcholanthrene by factors of 22 and 28, respectively. In vivo administration of the P-450 inhibitors diethyldithiocarbamate, SKF 525A, metyrapon, and pyrazol at doses from 50-150 mg/kg decreased lipid peroxidation by over 90%. As a P-448-directed inhibitor, 100 mg/kg α-naphthoflavone diminished the ethane exhalation rate by 97% in benzo (α)pyrene-induced mice, whereas SKF 525 A had no effect. Hepatic glutathione peroxidase levels were decreased to below 5% of controls by dietary selenium deficiency or by feeding

the animals with 800 ppm AG^+. Se-deficient mice injected with 400 mg/kg diethylmaleate plus 100 mg/kg paracetamol (alternatively 100 mg/kg aminoantipyrine) evolved a mean of 290 (150) nmoles of ethane/kg hr compared to 50 (7) nmoles hr in Se-adequate animals. Diethylmaleate plus 80 mg/kg ethylmorphine lead to exhalation of 85 nmoles/kg hr in silver acetate-treated mice. The following conclusions were drawn: (1) independent of the nature of the substrate drug, biotransformation is accompanied by lipid peroxidation; (2) the glutathione peroxidase system effectively protects against this lipid peroxidation: pathophysiological consequences are not manifest unless enzyme and substrate levels are drastically decreased; (3) it is proposed that the microsomal monoxygenase is the source of the activated oxygen species that initiates lipid peroxidation.

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THE EFFECT OF LIPOXIDASE INACTIVATION ON THE QUALITY OF EXTRACTED SOYBEAN OIL AND MEAL. R.D. Rice, 53 West Leys Road, Swanland, Near Hull, North Humberside, United Kingdom, and L.S. Wei, M.P. Steinberg, and A.I. Nelson, Dept. of Fd. Sci., University of Illinois.

It has been known for some years now that the beany or painty flavors characteristic of raw soybeans is due to the action of the enzyme lipoxidase, or lipoxygenase. The effect of inactivating lipoxidase, on the quality and flavor of full fat soya products has already been demonstrated, but until now, no work has been published on the application of these ideas to solvent extraction. During solvent extraction, conditions arise which allow enzyme activity. After flaking the conditioned meats, the flakes have a smell which is typical of lipoxidase catalysed oxidation of linoleic acid. The significance of this period of time, during which conditions remain suitable for lipoxidase activity, was the object of the investigation reported here. It was theorized that because of the intense flavor and odor of some of the known breakdown products of linoleic acid, the prevention of lipoxidase activity prior to extraction might result in an extracted meal of superior flavor. Similar reasoning, except based on free radical formation, suggested that the oxidative stability of the extracted oil might also be improved by inactivation of lipoxidase prior to oil extraction. Accordingly, experiments were carried out, in which soybeans were heated at various moisture contents and times, in steam, to inactivate lipoxidase. Once the optimal conditions had been determined, heat-treated and raw beans were extracted in a laboratory extraction system designed to simulate conditions in commercial extraction operations. The meal and oil obtained from these experiments was evaluated by a number of techniques in an attempt to determine whether or not any improvement had occurred. Oxidative stability in the oil from heat-treated beans was increased, as determined by the Swift Stability test, and by an organoleptic method. Similarly, blandness ratings of the heat treated meal were also superior to the meal produced from raw beans.

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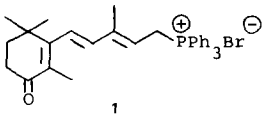
APPROACHES TO THE SYNTHESIS OF CANTHAXANTHIN. M. Rosenberger, P. McDougal, G. Saucy, and J. Bahr, Hoffmann-La Roche Inc., Bldg. 76, Room 1102, 340 Kingsland St., Nutley, NJ 07110.

Some new syntheses of canthaxanthin will be described. The basic construction involves the coupling of a C_{15} phosphonium salt **1** with the dialdehyde **2**, and the synthetic efforts center about the formation of the salt **1**. Several routes have been followed based on trimethylcyclohexenone **3**, the diketone **4** and α-ionone **5**.



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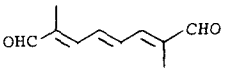

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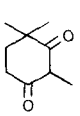

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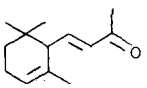

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CAROTENOIDS OF THE CAPE ROCK LOBSTER AND THEIR UTILIZATION. A.A. Spark, Susan Peall, K. Damstra, Sandra Rudd and Ann Templar, Fishing Industry Research Institute, University of Cape Town, Private Bag. RONDEBOSCH 7700, South Africa.

The Cape Rock Lobster, *Jasus lalandii*, one of the delicacies of the lobester and crab fisheries, is in great demand overseas. Mostly, these lobsters are packed as frozen tails, which generates a large amount of waste product in the form of bodies. Some of these are processed for leg meat but most are discarded. The carapace in particular is heavily pigmented. The pigments in the shell comprise astaxanthin and its diester, canthaxanthin, zeaxanthin, β-carotene, (lutein), and echinenone, in decreasing order of abundance. Failure to find or identify α-doradexanthin makes the identification of lutein suspect at this stage as this is one biochemical pathway to astaxanthin. The major part of the diet of rock lobster is the black mussell *Choromytilus meridionalis*, which is thought to be the source of the carotenoid skeleton as a presently unidentified xanthophyll. The rock lobster is capable of large-scale mobilization of carotenoids during moulting, and there is strong evidence that it can synthesize astaxanthin from β-carotene. Commercial interest in the lobster pigments centers on the feeding of trout grown in sea water. Extracts of shell have been shown to give the required pigmentation, and work in hand is aimed at making concentration of pigment economically feasible.

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WATER DISPERSIBLE CAROTENOID PRODUCTS. Nadav Ben-Eliezer, Bulk Specialties-Development Department, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110.

The chemical stability of various β-carotene powders was evaluated. A model system was designed to study the reaction kinetics of color fading of β-carotene in beverages. This included color measurement of samples exposed to elevated temperatures as well as sunlight, and comparing this with the stability of the beverage stored at room temperature. The results indicate that the stability of β-carotene may be ascertained by the above model.

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NEW APPLICATIONS OF CAROTENOIDS IN FAT BASED SYSTEMS. A.A. Metzner, 340 Kingsland Street, Nutley, NJ 07110.

Many manufacturers are producing light foods by reducing the fat content in order to reduce calories. These systems present challenges to using carotenoids for coloring diet salad dressings, imitation cheese, diet spreads, etc. Carotenoids can be used in an oil spray to color the exterior of extruded cereal grain products. Carotenoid colors added to the oil phase of spray-dried cheese or imitation dairy products have been successful. Beverages (still and carbonated) can be colored with carotenoids by adding the color to the flavor oil and homogenizing both into the syrup concentrate. Applicable market forms, use levels, and stability data of the carotenoids are discussed.

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CAROTENOIDS OF THE SPONGE *MICROCIONA PROLIFERA*: IMPLICATIONS FOR THE MARINE FOOD WEB. Carter Litchfield, Biochemistry Department, Rutgers University, New Brunswick, NJ 08903, and Synnøve Liaaen-Jensen, Norwegian Institute of Technology.

The ten main carotenoids of the marine sponge *Microciona prolifera* have been identified by chromatographic and spectrophotometric techniques. Three carotenoids with new structures were found: 3,4(2,3)-didehydro-γ,χ-carotene, 3-hydroxy-κ,φ-carotene-6,8-dione (trikentriophidin), and 7,8-didehydro-β,β-caroten-3-ol (allobetaxanthin). Clathriaxanthin (41%), trikentriophidin (14%), and trikentriorhodin (13%) are the major carotenoids of this sponge. β,β-carotene, allopurpurin (=tedaniaxanthin), alloxanthin, β,ε-carotene, 3,4(2,3)-didehydro-γ,χ-carotene, crocoxanthin, and allobetaxanthin are also present. All carotenols occur as esters. The unique carotenoid pattern in *Microciona prolifera* offers the best evidence to date for the aromatization of algal carotenoids by a sponge. This implies a direct algae-to-sponge link in the marine food web.

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THE OIL DROPLET CAROTENOIDS OF THE EYES OF BIRDS AND REPTILES. Brian H. Davies, Susan Polland, and Rosemary J. deB. Apps, Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Penglais, Aberystwyth, Dyfed, SY23 3DD, United Kingdom.

The individual color-sensitive cone cells of avian retinas, like those of reptilian retinas, each contain a large oil droplet through which light has to pass on its way to the photoreceptors. These droplets are intensely colored (red, yellow-orange or yellow-green) because of the presence of up to molar concentrations of different carotenoids. The coloured droplets act as light filters to improve color discrimination and visual acuity. Some 40 years ago, George Wald initiated studies on the nature of these carotenoids—studies which have continued in other laboratories and which have now been completed, together with an investigation of the metabolic origin of the pigments, in our own. It is clear that the yellow retinal oil droplets xanthophylls, lutein and zeaxanthin, are of dietary origin and that zeaxanthin can be converted either into the red astaxanthin (by the insertion of conjugated keto groups, thus lengthening the chromophore) or, by oxidative degradation (thus shortening the chromophore), into Wald's greenish-yellow "galloxanthin," now shown to be 10'-apo-β-carotene-3,10'-diol. Both transformations are readily explicable in terms of well-established pathways of carotenoid metabolism in animals. Much more difficult

to explain is the presence in the avian retinas of the pale-yellow ϵ-carotene, as their sole carotene hydrocarbon, constituting some 15% of the total pigment. This rare carotene is not of dietary origin, nor is it present in egg yolks. Any attempt to account for its presence seems to negate at least one of the fundamental and long-held tenets of carotenoid metabolism. Our studies, now extended to reptilian retinas, continue with the support of the Science Research Council.

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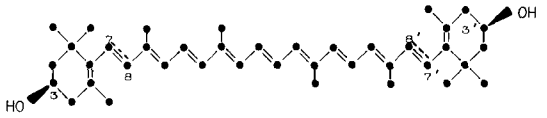
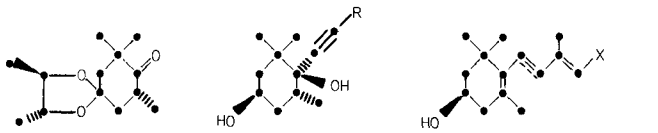
A NEW APPROACH TO THE SYNTHESIS OF OPTICALLY ACTIVE ZEAXANTHIN AND ALLOXANTHIN. Giuseppe Weber and Gabriel Saucy, Hoffmann-LaRoche, Chemical Research Department, Nutley, NJ 07110.

A new approach to produce the optically active synthon *4* via the monoketal *3* will be presented. The synthesis of optically active Zeaxanthin (1) and Alloxanthin (2), based on the successful preparation of (5) from (4), will be discussed in detail.



1 Zeaxanthin (all-*trans*, 3R,3'R-Dihydroxy-β,β-carotene)
2 Alloxanthin (7,7',8,8'-bisdehydro derivative)



3 4 5 (X=CHO, $CH_2P\emptyset_3Br$)

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CAROTENOID-PROTEIN COMPLEXES. George Britton, Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, United Kingdom.

Free carotenoids are yellow, orange, or red lipids, insoluble in water and unstable in light. In tissues of many invertebrate animals the carotenoids exist as specific complexes with protein. These carotenoproteins are normally water soluble and stable even in sunlight. The binding to protein frequently results in a large hypsochromic shift (100–150 nm) in the light absorption spectrum, to give a different range of colours. Well known examples are crustacyanin (λ_{max} 630 nm) , the blue pigment of the lobster *Homarus gammarus*, and other purple-blue pigments from the chondrophore *Velella velella* (λ_{max} 600 nm) and the starfish *Asterias rubens* (λ_{max} 570 nm), and the red ovorubin (λ_{max} 510, 545 nm) from eggs of the snail *Pomacea canaliculata*. In all these cases the carotenoid prosthetic group is astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) or its 7,8- and 7,8,7',8'-dehydro derivatives (λ_{max} 470-490 nm). Model carotenoproteins may be produced by combination of the colourless apoprotein with a range of different carotenoids. The specificity of binding in red ovorubin complexes differs greatly from that in the blue complexes, e.g., ovorubin selectively binds only the (3*S*, 3'*S*) isomer of astaxanthin, whereas the other proteins will accept (3*R*, 3'*R*)-, (3*R*, 3'*S*)- and (3*S*, 3'*S*)-astaxanthin almost equally well. The properties of some natural carotenoproteins will be outlined and results of binding studies and physico-chemical investigations of natural and model reconstituted carotenoproteins will be discussed in relation to the possible mode of carotenoid-protein binding and the mechanism of the spectral shift.

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SOLAR AUGMENTED SOYBEAN DRYING. Bill R. Hall, Technical Services, Inc., Helton Drive–Industrial Pk., Florence, AL 35630.

The objective of this overall project was to provide for the analysis, design, fabrication, and demonstration of a solar energy system for process drying of soybeans. The system consists of an array of 672 air collectors that preheat the inlet air to existing continuous flow dryers at the Gold Kist Soy facility at Decatur, Alabama. This experimental system, sponsored by DOE, has been operational since June 1, 1978. Due to soybean process equipment maintenance, a system utilization of only 46.4% was achieved. The 1,215 m^2 (13,104 ft^2) system delivered 0.867TJ (822.5 x 10^6 BTU), or 1.3% of the energy requirement for one dryer in the first year of operation. This paper, oriented to Phase III, Performance Evaluation, will describe the facility, the first year of operation and present performance operational, and life cycle cost analyses.

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EFFECTIVE ENERGY MANAGEMENT. D. Harrelson and D. Antonellis, The Foxboro Co., Foxboro, MA 02035.

OPEC, the spot oil market, our awakening neighboring countries. . . who would have thought a decade ago that these seemingly docile, insignificant, and unheard of factions and factors would weigh heavily on the performance of the United States industrial complex. But they are! Even more freightening is their expected future impact: THE FUTURE SUPPLY OF CRUCIAL ENERGY WILL BE TIGHTER, AND WILL COST MUCH MORE. The cost of processed goods will continue to escalate as the energy required for their processing soars. It is precisely for this reason that industry, to be competitive both nationally and internationally, must reevaluate its energy usage: INDUSTRY MUST INCREASE ITS PROCESS EFFICIENCY. This can be accomplished within the scope of a comprehensive Energy Management Program that addresses both the process and utility areas of industrial plants. But energy management, like the energy dilemma itself, is relatively new, and, therefore, has assumed a mystique that is oftentimes. unnecessarily self-discouraging. ENERGY MANAGEMENT OFFERS A VIABLE SOLUTION TO IMPROVED PROCESS AND UTILITIES ENERGY UTILIZATION. A general approach to more accurate energy measurements, improved process and utility plant equipment control, and in integrated Energy Management Program will be presented. This approach will build upon a basic "starting" program and develop it through a complete Energy Management System. Specific examples of strategies and techniques employing both analog and digital technology will be presented for both the utility and process areas.

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UTILITY SERVICES COSTS. Ralph Aletmus, Davy McKee Corporation, 10 So. Riverside Plaza, Chicago, IL 60606.

(1) Selection of electrical utility rates for lowest power cost based on an evaluation of voltage requirements, load factors, demand, and energy usage. Negotiation of electrical utility contracts outside published rates. (2) Conversion to alternate fuels for process and steam generation. Feasibility of using low or medium BTU gas. (3) Impact of fuel cost increases and electrical rate increases on the economics of today's facilities upgrading projects.

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CORPORATE ENERGY CONSERVATION AND COMPLIANCE PROGRAM: A CASE STUDY. T.C. O'Connor, Bunge Corporation, P.O. Box 1987, West Memphis, AR 72301.

What I intend to discuss in the 20 minutes allotted to me is the development to date of the energy conservation program in Bunge Corporation as it applies to the Soybean Processing Division. I intend to cover three specific areas: (A) How the program was initiated. The successes that we had and also the failures. Within this context I specifically cover how people reacted to the program, instituting a corporate energy reporting system, and implementing individual plant conservation programs. (B) The effect of government regulations. This will specifically deal with the Natural Gas Policy Act and the National Energy Act and how they affect those industries with SIC 20. I will cover such topics as disseminating the information to plant personnel and the problems in obtaining feedback. (C) Conducting energy audits. This will be explored from the standpoint of hiring outside consultants to do the work versus having in-house personnel conduct the audit. I don't intend to cover what's actually entailed in an audit, except in a broad way. I will also cover getting plant personnel cooperation in conducting the audit, and implementing the recommendations. Also, the tax effects of installing energy saving devices will be covered.

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PROCESS CONSIDERATIONS–ENERGY CONSERVATION IN SOLVENT EXTRACTION. Kenneth W. Becker, Davy McKee Corporation, 10 So. Riverside Plaza, Chicago, IL 60606.

Energy conservation in oilseed solvent extraction operations is essential to stay in business. With higher and higher energy costs, the overall plant design and the process design of individual equipment systems must be carefully controlled to prevent prohibitively high steam and solvent costs. A plant design that was good in the early 1970s will no longer be adequate for the 1980s. For example, excellent solvent drainage must be achieved; start-up solvent losses must be minimized far below those experienced in most plants; and mineral oil absorption systems must be designed that will operate at top-notch efficiency year after year with minimum maintenance and operating difficulties. Very high or even moderately high solvent loss can take away most, if not all, a company's profits. It can also cause significant difficulties with air pollution regulatory agencies or prohibit the installation of a new plant altogether.

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CONSIDERATIONS ON ENERGY SAVING IN VEGETABLE OIL REFINING. Anthony Athanassiadis, Extraction De Smet S.A., 265, Prins Boudewijnlaan-B-2520 Edegem, Belgium

The vital importance presently gained by energy consumption in the processing cost of refined oils need not be emphasized. Saving energy, or even using it under its least expensive form, should now

be a major concern for every oilmiller. This is why recovery of excess heat, dissipated until now, as well as reduction of consumptions–especially steam consumption for the refining process–have become prime targets in design of the equipment used. In the present survey, we develop certain energy saving techniques which we have been practicing for several years, but also some other, recently experimented procedures. These measures have enabled us to reduce by half the steam and fuel consumptions in an existing refining plant composed of equipment generally designated as "classical" up to the present date.

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SIMPLIFIED MISCELLA REFINERY FOR ENERGY SAVING IN COTTONSEED OIL. Alfredo Garcia-Serrato, Los Molinos, S.A., Sonora, Mexico.

Distinctive pigments and quick deterioration of cottonseed oil long made one-pass refining difficult if light color and low loss were desired. The development of miscella refining of this oil at the extraction plant site made it possible to have a success operation with once refined cottonseed oil, particularly with high free-fatty-acid contents. The mixing of miscella and caustic for the neutralization reaction is difficult because these liquids are immiscible and uniform contact must be obtained mechanically. Diverse equipment is employed at miscella refineries to heat, cool, and mix miscella and caustic solutions in order to obtain an intimate mixture for full reaction. By employing a battery of holding vessels and by utilizing the heat from the extraction plant and soapstock desolventization, this operation can be simplified and uniform conditions can be maintained, saving energy and permitting better control with good results while refining cottonseed oil ranging from 1.5 to 6.0% free-fatty acid.

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CELLULAR AGING. Donald W. King, and Gerda Nette, Columbia University, New York, NY 10032.

One of the principal problems in aging has been the controversy on whether aging is a distinct disease, or merely a summation of many disease processes resulting from environmental insult over a period of several decades. The definitive lifespan of several different species indicates a strong genetic factor in the aging process. Although many diseases have been associated with aging, such as atherosclerosis, hypertension, adult-onset diabetes, and neoplasia, only a few diseases are specifically associated with aging populations, e.g., osteoarthritis and osteoporosis. Theories of aging have concentrated on extracellular changes in the ground substance matrix of vessels and other tissues, generalized cellular degeneration including loss of cells (particularly the CNS), and diminished function in special regulatory cell systems, including the neuroendocrine and the immune systems. Our studies have concentrated on intrinsic cellular changes. Utilizing cells from young and old individuals grown in tissue culture, a series of experiments with hybrid, cybrid, and reconstituted cells have been performed. Studies of growth curves, generation times, and thymidine uptake have shown that although the young nucleus has a significant rejuvenating effect on old nuclei in hybrid cells, the cytoplasm of young, rapidly dividing cells also has a stimulatory influence on the old nucleus. This suggests that proteins or other materials in the cytoplasm are capable of de-repressing nuclear genes.

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HISTOLOGICAL FINDINGS RELATED TO LIPID DEPOSITION IN AGING TISSUES. A. Whitley Branwood, College of Physicians & Surgeons, 630 West 168 Street, New York, NY 10032.

The accumulation of lipids in tissues has been regarded as a generalized aging process in man because it occurs in both vascular and many nonvascular tissues throughout the body. Histological evidence of increased lipid accumulation has been noted in heart muscle, aortic wall, atherosclerotic plaques, skeletal muscle, and lymph nodes. A study was undertaken to assess histologically the accumulation of lipid in atherosclerotic plaques and other tissues from autopsy material in cohorts in an attempt to determine the relationship between this lipid accumulation and the age and sex of the individuals.

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AGING AND ARTERIOSCLEROSIS. Hans Kaunitz, Department of Pathology, Columbia University, 630 West 168 Street, New York, NY 10032.

During this century, arteriosclerosis has been discussed as a disorder of lipid metabolism, based largely on the belief that cholesterol has atherogenic properties. The recent interest in the processes of aging seems to change the prevailing theories about arteriosclerosis. Although lesions related to arteriosclerosis are present from infancy, its clinical symptoms occur at the same time as other age-dependent conditions, especially alterations of the immune systems which lead to various autoimmune diseases which are involved in the arteriosclerotic process. The changes seem to be related to the cells' genetic make-up, responsible for the "programmed" appearance of the signs of aging. Aging is associated with the gradual increase of the various lipid fraction (with the notable exception of the high density serum lipoproteins); this is evidently not the cause but the consequence of the alterations of the genetic system. Discussion is needed concerning whether the lipid changes accompanying aging (and arteriosclerosis) may delay the deteriorating effects of the genetic changes.

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RELATIONS BETWEEN NUTRITION, SERUM LIPIDS, AGING, AND ARTERIOSCLEROSIS. E. Renner, Department of Dairy Science, Justus-Liebig-University, Bismarckstr. 16, D-6300 Giessen, Federal Republic of Germany.

The incidence of arteriosclerotic complications (myocardial infarctions, coronary heart disease) increases with age; age can be called a risk factor. Most serum lipids (total cholesterol, very low density lipoproteins, low density lipoproteins, triglycerides) are elevated, but high density lipoproteins (HDL) are often reduced in aging. However, on the basis of such correlations, it is not justifiable to assume a causal relationship between the changes of the serum lipids and arteriosclerotic lesions. Some investigators ascribe "protective" properties to HDL; yet occasionally no significant decreases, but even increases have been reported in aging. In order to compare the relationship of arteriosclerosis to diet and blood lipids, age-adjusted data must be used. This should be accompanied by observations of blood pressure and other risk factors for the evaluation of the aging processes.

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CORRELATION OF DIETARY CHOLESTEROL WITH PLASMA TRIGLYCERIDES IN WOMEN. Grant G. Slater and Roslyn B. Alfin-Slater, University of California, Los Angeles, School of Public Health, Los Angeles, CA 90024.

The UCLA Diet Study is investigating possible correlations between dietary constituents and plasma lipids. A weekday, one-day diet (ODD) history and a seven-day diet (SDD) check list of cholesterol-containing foods were obtained from 402 women at UCLA, and were used to compute the amount of 17 selected nutrients and dietary cholesterol (DC) ingested. 350 women whose DC for SDD/7 was within ±400 mg/day of their ODD cholesterol were used. Fasting plasma samples were analyzed for total cholesterol (TC), HDL-cholesterol (HDL) and triglycerides (TG). The average DC was 323 mg/day for ODD and 375 for SDD. Average age, TC, HDL, and TG were 41 yr., and 190, 64, and 59 mg/dl, respectively. Plots of TG versus age for all subjects showed a slope of 0.99 mg/yr; however, those subjects consuming under 200 mg/day, 201–400, 401–600, 601–800, and above 800 mg/day of cholesterol had slopes of 0.97, 0.83, 0.81, 1.81 and 1.35 mg per year for ODD, while the SDD had slopes of 0.69, 0.99, 1.10, 1.56 and 0.61 mg/yr. The number of subjects in the dietary groups were ODD 126, 117, 67, 26 and 14, and SDD 50, 168, 106, 21 and 4. The average TG of these same groups were 58, 58, 60, 63 and 64 mg/dl from ODD and 59, 59, 59, 56 and 90 mg/dl from the SDD. Thus, using groups of subjects with daily cholesterol intakes between 150 and 800 mg, the average plasma TG does not change significantly as dietary cholesterol intake is increased; however, it appears that the TG/yr increases as dietary cholesterol, calculated from either ODD or SDD, increases. (Supported by the American Egg Board 1977–1979).

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IN VIVO LIPIDPEROXYDATION AND ORGANIC FREE RADICAL CONTENT OF RAT ADRENALS. EFFECTS OF ESSENTIAL FATTY ACID-, VITAMIN E-, AND SELENIUM DEFICIENCY. E.J. Christ, Unilever Research Laboratory Vlaardingen, P.O. Box 114, Vlaardingen, The Netherlands.

Lipidperoxidation in vivo has been identified as a basic deteriorative reaction in cellular mechanisms to the aging process. Some dietary factors controlling the rate and extent of the in vivo lipidperoxidation were studied. Rats on a diet deficient in vitamin E and Se for 13 weeks were clearly deficient and showed large increases in the in vivo lipidperoxidation as measured by ethane and pentane evolution in exhaled air, when compared to animals on a supplemented (control) diet. In contrast with these large differences, changes resulting from this dietary manipulation in free radical content of intact rat adrenals, were small and insignificant. The free radical content might have increased during the experiment (age?) in both dietary groups, with the increase being larger in the supplemented groups than in the deficient groups. The effect of feeding essential-fatty-acid-deficient rats varying levels of n-4, n-6, and n-7 acids for various periods of time has been studied with regard to the alkanes produced, as well as the fatty acid composition of liver phospholipids and liver triglycerides. It was found that the fatty acid composition of liver lipids depended on the nature and quantity of polyunsaturated fatty acid in the diet. The composition of the alkanes produced corresponded closely to the fatty acid composition of the liver phospholipids.

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CONSEQUENCES OF NUTRITION AND LIPID PEROXIDATION TO THE AGING PROCESS. Jon J. Kabara, Michigan State University, Department of Biomechanics, A419 East Fee Hall, East Lansing, MI 48824.

Lipid peroxidation as a factor in aging, cancer, effect of air-pollution, and so on, is heavily supported by contemporary research and thinking. We are only now realizing that biological systems are specifically designed to control the rate of oxygen utilization in much the manner of an engine using gasoline. Beyond the use of oxygen to sustain life, it can also be converted to a number of transient free radicals thought to be responsible for producing irreversible damage to membrane lipids and other biomolecules. It is the former reactant which we will deal with in our discussion. Polyunsaturated fatty acids (PUFA) are a prime target and form highly reactive lipid peroxides. This process, which leads to autoxidation, is self-perpetuating in the presence of oxygen. Were it not for unique biochemical systems and chemicals available to scavenge the formed free radicals, our biological systems would self-destruct. The consequences of the lipid peroxidation theory suggest modifications to our diet in order to avoid this "aging process." The theory becomes a molecular focal point to test and examine nutritional effects on the aging process. The mechanism of free radical damage in the pathogenesis of senescence and other disease processes refutes the present advocation of diets high in PUFA and makes a more formidable role for specific proteins in our diet.

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EDIBLE OIL PRODUCTS FROM LOW-ERUCIC-ACID RAPESEED OIL. B.F. Teasdale, Research Centre, 2211 St. Clair Ave., W., Toronto, Ontario, Canada M6N 1K4.

The USDA forecast for 1979-80 world production of rapeseed is 11.6 million tons. Although this amount is less than 25% of that for soybeans, rapeseed represents a substantial contribution to the supply of edible oils, particularly because of its adaptability to various climatic conditions. In quite a number of countries, for example, Sweden, Poland, Germany, and Canada, RS is the principal edible-oil crop. In Canada, low-erucic RS oil has become the largest single vegetable oil used in edible oil products; its usage exceeds even that of SB oil. Data will be given on the utilization of this oil and on the characteristics of the new Canola oil which is obtained from "double-low" rapeseed varieties (low in erucic acid and in glucosinolates).

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EFFECTS OF OIL EXTRACTION TECHNOLOGY ON RAPESEED MEAL QUALITY. E.H. Unger and S.J. Campbell, United Oilseed Products Ltd., Lloydminster, Alberta, Canada S9V 1K5, and D.I. McGregor, Agriculture Canada Research Station, 107 Science Crescent, Saskatoon, Saskatchewan.

To date, economic factors have dictated that rapeseed processors concentrate their major efforts on the production of a high quality oil. With the recent development of low glucosinolate rapeseed cultivars (i.e., Tower and Candle) containing 3 mg/g glucosinolates, consumption of RSM in Canada has increased rapidly, reflecting the improvements in feeding value. To further improve meal quality, investigations were undertaken to assess the effects of commercial oil extraction on RSM quality. In this paper, effects of cooking, extraction, and desolventizing on RSM quality were examined, including the changes in glucosinolate content, enzyme activity, amino acid composition, protein solubility, and available lysine. Protein denaturing and reductions in the availability of lysine were most significant in the desolventizing-toasting process.

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DEVELOPMENT OF A DEHULLING PROCESS TO IMPROVE THE CAKE-QUALITY. J.-J. Baudet, CETIOM, 174, Avenue Victor-Hugo, 75116 Paris, France.

Presently, rapeseed cake is little used in rations for monogastric animals (swines, poultry) despite an excellent amino acid ratio. As a matter of fact, the high cellulose content in the hull of the rapeseed seed leads to a decrease in the protein content of the cake and a smaller nitrogen and energy consumption. The experimental studies which have been carried out by the CETIOM for several years on the composition of rapeseed seeds have shown that dehulling greatly improves the cake composition, since it is possible to increase the protein content by 8 points with a residual cellulose content of 6%. These results justify the interest of rapeseed dehulling. That is why the CETIOM, the "Centre Stéphanois de Recherches Mécaniques (Hydromécaniques et Frottement)," and the "Groupe d'Huilerie OLEAGRI" have combined their efforts for two years to develop a dehulling process in industrial conditions. Two pilot-lines for dehulling and sorting rapeseed with an output of 3,5 T/h of seed were tested simultaneously in a French oil-refinery. Each line was provided with a different dehulling system: a CETIOM-registered system, and a H.E.F. (Hydromécanique et Frottement)-registered system. Nevertheless, both have the same sorting system for dehulled rapeseed on a fluidified bed (H.E.F.-registered system). The first results obtained in 1979 with these industrial devices suggested it is possible to produce a cake containing no more than 8% cellulose and 43% protein content. The authors will describe the output of the pilot-lines, the characteristics of the products obtained after dehulling, and the nutritional results obtained with this new dehulled rapeseed cake.

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REVIEW OF RAPESEED MEAL IN ANIMAL NUTRITION, PART 1. RUMINANT ANIMALS. Sigvard Thomke, Department of Animal Husbandry, Swed. Univ. Agricultural Sciences, S-750 07, Uppsala, Sweden.

During recent years, world literature in this area has (increasing-by dealt with the assessment of low glucosinolate RSM-types (LGRSM). Generally, the LGRSM-types show clear advantages over the high glucosinolate RSM-types (HGRSM). Acceptibility of concentrates to dairy cattle may be influenced negatively by increasing RSM of commercial Canadian type beyond 15%. LGRSM seems to be acceptable up to at least 20% of the concentrate mixture. LGRSM-type meal of cv. Candle may possibly still be accepted at higher levels. There are indications of a higher nutritive value of the LG vs. the HGRSM-types. Candle has been found to be as good as, if not better than, Tower RSM. HGRSM of European origin at a level of 15-20% of the concentrate seems to decrease milk production and to influence milk composition negatively. Canadian type LGRSM feeding at a level of 25% of the concentrate does not seem to be harmful to dairy cattle. Inclusion of 34% LGRSM lowered feed intake and milk yield. Higher milk yields are indicated when Candle is included, in comparison with Tower. Only a limited influence on milk composition (protein, fat, solids, non-fat) has been reported to result from RSM-feeding. Both HG- and LGRSM increase milk SCN^- and decrease milk-I contents; the latter, however, to a minor extent. ITC and OZT have not been found in detectable amounts in milk. Inclusion of "gums" at levels up to several times the amount corresponding to normal production has not been found to impair production traits of dairy cattle or growing cattle. Microbiological degradation of RSM in the rumen can be minimized by formaldehyde treatment, which has been reported to increase milk production. Rapeseed and rapeseed expeller seem to be prospective fat and protein sources to dairy cattle, especially in rations low in crude fat (<15 g dig. fat/kg milk). The inclusion of these products increases blood cholesterol and plasma glycerides. The iodine-number of milk fat is increased.

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RAPESEED MEAL IN ANIMAL NUTRITION. II NON-RUMINANT ANIMALS. D.R. Clandinin, Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada T6G 2E3.

The most important factor that has contributed to expansion in usage of rapeseed meal (RSM) in feeds for livestock and poultry in recent years has been the development of low-glucosinolate type rapeseed by Canadian and European plant breeders. Meal derived from this new type of rapeseed, while not differing in proximate and amino acid composition, contains only about one-eighth as much glucosinolates as older high glucosinolate type RSM. The new low-glucosinolate type RSM, known in Canada as Canola Meal, may be used in rations for starting, growing, and finishing swine at the 10% level, and in rations for breeding swine as the sole source of supplementary protein. In rations for starting and growing chickens and turkeys, the new low-glucosinolate type RSM has been used successfully at the 20% level of inclusion and in laying and breeding rations for chickens and turkeys at the 10% level. These usage levels represent an approximate doubling of the usage levels generally found satisfactory for high-glucosinolate type RSM.

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CHARACTERISTICS OF MYROSINASE IN POLISH VARIETIES OF RAPESEED. Halina Nowak Kozlowska, Institute of Food Engineering and Biotechnology, University of Agriculture and Technology, Olsztyn, Poland.

Isolation, purification, and characterization of selected physical, chemical, and catalytic properties of myrosinase in different varieties of rapeseed was researched. Also, the parameters were determined for myrosinase inactivation at different conditions of temperature (80, 90, 100 C), relative humidity (80, 90, 100°%), and time (5–60 min) for intact and flaked rapeseeds.

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POLYMORPHISM OF GLYCERIDES. Reuben O. Feuge, USDA, SEA, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Changes in crystal structure frequently are critical factors in the utilization of semisolid and solid glycerides, such as shortenings and confectionery fats. Some effects of polymorphic changes and

methods of identifying and measuring these changes are described. The known polymorphism of glycerides in edible fat products is summarized and categorized insofar as possible. Areas in which more information is desirable are identified. The stability of polymorphic forms and means of effecting polymorphic transformations are discussed.

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FRACTIONATION OF BUTTER BY CRYSTALLISATION WITHOUT SOLVENT. COMPLETE DETERMINATION OF GLYCERIDE STRUCTURE OF EACH FRACTION. Jean-Paul Wathelet, Claude Deroanne, and Michel Severin, Faculté des Sciences Agronomiques de l'Etat, Chimie générale et organique, B-5800 Gembloux, Belgium.

Butter was fractionated without solvent, and each fraction was completely analyzed. In the first part, we describe the fractionation. Afterwards, we point out difficulties which have been found in the past when chemical analysis of those complex fats has been made, and we show how we have avoided them especially, when triglycerides are determinated in function of their unsaturation (silver thin-layer chromatography) and in function of fatty acid esterified on the second position (pancreatic lipase). Complete glyceride structure of each fraction is calculated according to the new method we proposed in 1978 at the Brighton I.S.F. Congress, because the Van Der Wal hypothesis cannot be used in this particular case. The main triglycerides of butter are, in decreasing order: OPB, OPP, OOB, and PPB and those of solid fraction obtained at 22 C are: OPP, PPB, and StPP. Those of correspondent liquid fraction are: OPB, OOB, OMB and OPP.

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SOLVENT PARTITIONING OF FATS: 1. BUTTER FAT. Sergio Longhi and Francis E. Luddy,* 611 Orchard Way, Hatboro, PA.

Prior studies concerned with the solvent fractionation of butter fat have utilized extremely low temperatures and high solvent ratios to produce a myriad of fractions with widespread chemical and physical properties. These were quite useful in triglyceride composition studies, but did little to produce fractions with commercial potential. In contrast, Biocell has developed a relatively simple, solvent fractionation process which requires only modest solvent ratios and moderate crystallization temperatures. The process, which uses acetone or similar solvents, yields only three fractions of contrasting physical properties. The fractionation scheme and the analytical characteristics of the fractions will be presented and major emphasis will be devoted to the unique physical properties of the fractions.

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PREDICTION OF THE THERMAL BEHAVIOR OF FATS. Roger Perron, Centre National de la Recherche Scientifique, 2-8 Rue Henry Dunant, 94320-Thiais, France.

The study of the distribution of melting points of triglycerides in function of carbon number and unsaturation, and the comparison of DTA results on palm oil, rapeseed oil, and derivatives, led us to conceive a binary representation of fat melting. In this representation, a hyperethylenic compound is opposed to various triglycerides, each of them able to induce a crystalline phase on the cooling, anteriorly applied. Straight lines connect the melting temperatures of β and α phases of these triglycerides to those of the hyperethylenic compound calculated from equations established elsewhere. These lines are simplified representations of various supposed simple solid-liquid equilibria, with consideration given to the small differences between enthalpies of species, and improbable compound formation or solid phases separation with a hyperethylenic entity in the complex mixtures studied. Intersects of the isolog line related to the fat unsaturation, with the other lines, are taken as the various melting temperatures of solid phases. Thermometric previsions so obtained are presented for different fats and compared with DTA results. The prediction of thermal behavior is achieved by evaluating enthalpies. The problem is oversimplified by considering only the main crystallization inducers triglycerides, grouped according to their carbon numbers. The enthalpies of each group per gram of sample are calculated, and the corresponding averaged melting temperatures are deduced from the isolog intersects. Finally, these group enthalpies are reported on a graph at the appropriated temperatures, as proportional elongations of triangular figures, and the envelope curve is deduced and compared with experimental DTA curves. β' forms are also taken into account, and the method is applied on palm oil, rapeseed oil, and other fat products, and discussed.

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THERMAL BEHAVIOUR OF TRIGLYCERIDES. Roger Perron, Centre National de la Recherche Scientifique, 2-8 Rue Henry Dunant, 94320-Thiais, France.

Melting enthalpies of triglycerides are first considered. For unsaturated species, it is shown that these quantities not only depend linearly on the molecular carbon number (like saturated compounds) but also on the number Δ of double bonds per molecule, by an additional term, so that: $\Delta H = (an-b)3 + 27.5\,(e^{-k\Delta}-1)$, with $k = 0.706$. Good agreement between experimental and calculated enthalpies confirm that this exponential term remains valid whatever the carbon number or the crystalline form. The *a* and *b* coefficients, related respectively to chains and end groups interactions, are shown to depend on the crystalline forms and longitudinal molecular arrangements. The necessity of using reduced carbon numbers for oleic and linoleic chains in Timms's empirical equation is easily and accurately justified. Melting temperatures are then considered. Zacharis's equation is applied to various unsaturated triglycerides, and the deduced T_∞ (infinite chain m.p.) and Co (negative coefficient) are given and discussed. Comparison of the *c* values related to CH2 melting entropy calculated from *a* and T_∞ or *b* and Co, enable us to propose an equation for melting entropies, and consequently a general equation for melting temperatures of triglycerides.

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THE RELATIONSHIP BETWEEN SOLIDS CONTENT, AS DETERMINED BY NMR, DILATATION AND SOLID FAT INDEX. Th.J.H. Geurtz, Unilever Research Vlaardingen, PO Box 114, 3130 AC Vlaardingen, The Netherlands; D. Waddington, Unilever Research Colworth House, Sharnbrook, Bedford, United Kingdom.

In recent years, many workers have devoted considerable attention to the examination of nuclear magnetic resonance (NMR) techniques for the determination of the solids content in commercial fats and shortenings. Such studies have mainly been directed toward devising a more efficient and straightforward method to characterize the melting behavior of fats than the hitherto extensively used dilatation technique. The principles of these various NMR techniques will be briefly reviewed in relation to their use for the determination of the solid fat content in fats. The relationship between solids content and dilatation value will be discussed and particular attention will be given to a discussion of the term "Solid Fat Index." Ringtest results will be presented showing the successful application of the method in a number of factory and research laboratories.

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PULSED NMR METHOD FOR DETERMINING SOLID FAT CONTENT: COLLABORATIVE STUDY. Jack H. Mellema, Bryan L. Madison, and Robert G. Manning, Kraft, Inc. R&D, 801 Waukegan Road, Glenview, IL 60025.

Attempts were made to determine the solid fat content (SFC) of edible oils using pulsed nuclear magnetic resonance in order to replace the existing dilatometric solid fat index (SFI) method, which is tedious and time consuming. A collaborative study was carried out among users of the Praxis SFC-900 Pulsed NMR in order to note the differences between the two methods. Instrumental calibration was achieved using mixtures of tristearin and olive oil of known solid fat content. Four groups of oils representating margarine oils, hydrogenated shortening, palm oils, and confectioners fat, varying in hardness from 10% to 75% (at 10.0 C), were tested by 14 collaborators. Approximate least square equations were proposed to define differences between the SFI and SFC readings.

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IDENTIFICATION OF STEROIDS BY CHEMICAL IONIZATION MASS SPECTROMETRY. Yong Y. Lin, Department of Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX 77550.

Mass spectrometry has become one of the most important techniques for the analysis of steroids. Although structural identification and elucidation of fragmentation processes of steroids by electron-impact ionization (EI) is well established, the mass spectrometry conducted by the more gentle process of chemical ionization (CI) offers several advantages that provide structural information unattainable by the EI process. Mass spectrometric analysis of various natural and synthetic steroids have been carried out in our laboratory using methane, isobutane, ammonia, trideuterioammonia, and hydroxyl anion as reagent gases. The CI spectra of steroids, in general, give simple and well characterized ions which are easy to interpret (Table 1).

The mild ionization process of CI ionizes the intact steroid molecules, resulting in molecular weight information. On the other hand, structural informations can be obtained by varying the experimental parameters, such as ionization temperature or reagent gases. The possibility of ion-molecule reaction between the reagent gas and individual functional groups of a compound of interest is another promising aspect of CI operation. The most gentle process of ammonia CI is also the most instructive. The ion-molecule reaction can be carried out with a wide scope of selectivity among functional groups, which permits the recognition of functional groups and some stereochemistry. Trideuterioammonia exchanges rapidly with active hydrogens (OH, SH, COOH, NH_2, etc.) in steroid molecules in the CI reaction, thus providing a convenient means of active hydro-

gen determination by mass spectrometry. Utilization of various chemical ionization techniques for the analysis of steroids and their conjugates will be discussed.

TABLE 1

Reagent Gases	Major ions
CH_4 or i–C_4H_{10}	Adduct ions($M+C_2H_5^+$ and $M+C_3H_5^+$, or $M+C_4H_9^+$); Protonation $(M+H)^+$; Hydride abstraction $(M-H)^+$; Eliminations $(M-X)^+$; etc.
NH_3 (or ND_3)*	Adduct ions $(M+NH_4^+)$; Protonation $(M+H)^+$; Substitution $(M+NH_3-X)^+$; Eliminations $(M-X)^+$; etc.
OH^-	Proton abstraction $(M-H)^-$; Eliminations $(M-X)^-$; etc.

* Deuterium exchange occurs with all active hydrogens in steroids.

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STEROL ANALYSES ON CITRUS, LEAF PROTEIN CONCENTRATES AND MYCORRHIZAL FUNGI. Harold E. Nordby, U.S. Citrus & Subtropical Products Laboratory, 600 Avenue S, N.W., P.O. Box 1909, Winter Haven, FL 33880.

Phytosterols are present in citrus juice as 4,4-dimethyl, 4α-monomethyl, and desmethyls in four fractions vis, free sterols, sterol esters, sterol glucosides and esterified sterol glucosides. Desmethyls in the four fractions were analyzed by GLC as TMS derivatives. Sterol esters subjected to $AgNO_3$-TLC along with GLC revealed long chain fatty acids (C_{20}-C_{29}) to be associated mainly with the 4,4-dimethyls. Minor desmethyl sterols were detected in citrus juice when the sterol fraction was subjected to $AgNO_3$ column chromatography. A survey of 12 new GLC liquid phases to determine resolution of "critical pairs" of sterol acetates was undertaken. Several of these phases were also shown to resolve free sterols before and after hydrogenation. Desmethyl sterol profiles change with maturation of the citrus fruit. Profiles of desmethyl sterols in roots of citrus infected with *Glomus* mycorrhizal fungus were different from non-infected roots, campesterol being the major sterol in the fungus itself. Lipids from leaf protein concentrates have unusual desmethyl sterol profiles.

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CONFORMATIONAL ANALYSIS OF STEROIDS: COMPARISON OF X-RAY CRYSTALLOGRAPHIC OBSERVATIONS WITH DATA FROM OTHER SOURCES. William L. Duax, Medical Foundation of Buffalo, Inc. 73 High Street, Buffalo, NY 14203.

Crystallographic data on over 400 steroids collected in the *Atlas of Steroid Structure* provide information concerning preferred conformations, relative stabilities, and substituent influence on the interactive potential of steroid hormones. Preliminary analysis of these data indicates that observed conformational details and directionality of hydrogen bond formation are intramolecularly controlled, and the influence of crystal packing forces is negligible. The crystallographic findings are being compared with theoretical calculations and data on receptor binding and biological activity. *17β-Side Chain Orientation:* A comparison of the crystallographically observed conformations of the 17β-side chain of 67 pregn-20-ones with force field calculations for 15 such structures indicates that the magnitudes of errors in the calculated orientations of the 17β-side chains are from two to seven times greater than the perturbations due to crystal packing. *A-Ring Conformation:* The Δ^4-3-one ring, common to active progestins and corticoids, normally has a conformation midway between the 1α,2β-half chair and the 1α-sofa forms, but strain introduced into the molecule by substitution on the fused ring system is observed to shift the A-ring conformation toward the more symmetric forms with an attendant change in the conjugation of the 4-ene-3-one system. Introduction of an additional double bond at C(9)-C(10) causes the A-ring to adopt an inverted chair form. Because many of the steroids with inverted A-rings are among the highest affinity binders to the progestin receptor, it has been proposed that the inverted conformation is optimal for such binding. *Hydrogen Bonding:* The pKa's of estrone and estradiol in methanol differ by 0.10 pK units. In the solid state the more acidic estrone freely contributes its hydrogen atom to form a single hydrogen bond. Estradiol, however, always acts as a hydrogen bond donor and acceptor, thus retaining a full hydrogen of its own. The observed differences in pK values and hydrogen bond patterns of steroids having high and low affinities for the estrogen receptor suggest that the C(3) hydroxyl probably acts as a hydrogen bond donor and acceptor in the active site. Theoretical calculations indicate that a sequential pair of hydrogen bonds may have an energy of 10 Kcal/mole. Research supported by grant no. CA-10906 from the National Cancer Institute, DHEW.

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CHOLESTEROL METHODOLOGY FOR HUMAN STUDIES. Bennie Zak, School of Medicine, Department of Pathology, Wayne State University, Detroit, MI 48201.

The explosion in methodological advances in clinical chemistry laboratories has not failed to include the determination of serum cholesterol. Its value as a serum determinand has been enhanced considerably by the attention given to the strong emphasis on the role that lipids may play in cardiovascular problems. The present discussion will attempt to demonstrate the evolution of cholesterol methodology, from gravimetric through the strong acid systems involving the Liebermann-Burchard and iron reactions to the more modern enzyme reagent systems using equilibrium or kinetic approaches with colorimetric, fluorimetric, or electrometric detection. Manual and automated approaches using monochromatic and dual wavelength technology will be considered, including spectophotometric application to electrophoretic separation systems. The total discussion will include both methodological classifications as well as analytical considerations such as interferences, blanking (both static and dynamic) and standardization, including the various ways to calibrate the procedural phases one might select for clinical use. Some descriptions of the different end point reactions will be attempted in terms of intensive properties of reactions as exemplified by molar absorptivities and the relationship of these properties to the manner in which samples are handled, and to the manner the concentration of the determinand of the sample is measured.

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THE ANALYSIS OF URINARY STEROIDS. Per Vestergaard, Rockland Research Institute, Bldg. 37, Orangeburg, NY 10962.

The last decade has seen a great shift in focus in the study of the metabolism of hormonal steroids towards the assay of blood steroids and away from urinary steroid determination. This is largely due to the impact of radioimmunoassay methodology for steroids and the ease and convenience of this technique for single substance determination in blood. Nevertheless, it is as true today as when it was stated by G.F. Marrian at a keynote address at the third International Congress of Biochemistry in the fifties, that "blood and urine determinations (of steroids) should not be regarded as alternatives, whose relative merits can be argued about, but as sources of different kinds of information which may be supplementary to each other." The assay of urinary steroids remains an important source of information about the metabolism of steroids. Early methodology consisted mainly in group assays for urinary steroids, and this approach survives today, although used less and less, in clinical laboratories. The trend has otherwise been towards the assay after isolation, usually chromatographic, of individual steroids. Current methodology for the estimation of urinary steroids will be surveyed, with particular emphasis on techniques aimed at determining a broad spectrum of steroids. The multi-column, medium pressure, liquid chromatographic procedure developed in the author's laboratory will be compared with other methodology in this area.

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DETERMINATION OF MOLECULAR FORMULA AND STEREOCONFIGURATION BY X-RAY DIFFRACTION ANALYSIS. Jerome Karle, Code 6030, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375.

The development of methods for structure determination by x-ray crystal structure analysis has provided a valuable tool for investigations of broad classes of compounds. The immediate information obtained is atomic arrangement and, when required, atomic identification. This information affords deeper insights into such matters as reaction mechanisms and synthetic processes that find broad application in the investigations of steroids. Steroids often present a challenge to the methods of crystal structure analysis because they usually crystallize as almost equal atom (ignoring hydrogen) structures in noncentrosymmetric space groups, the most difficult type of problem to analyze. Several such structures have been investigated in our laboratory, some of which had additional peculiarities that gave rise to special problems. Particular examples concern a toxin of quite unusual physiological properties and plant growth regulators that act in nanogram quantities per plant. It is planned to outline some aspects and characteristics of crystal structure analysis using these substances and others as examples.

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IDENTIFICATION OF STEROLS AND BILE ACIDS BY COMPUTERIZED GAS CHROMATOGRAPHY-MASS SPECTROMETRY. William H. Elliott, Department of Biochemistry, St. Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104.

Ideally, the ability of the gas chromatograph to separate isomeric sterols or bile acids provides the mass spectrometer with individual substances whose mass spectral fragmentation patterns are recognizable by the experienced analyst. Data collected by the computer

not only may be visualized rapidly as typical mass spectra of these separated components, but chromatograms of a number of selected fragment ions eluted coincident with the total ion current can be provided to establish the identity of such components. Typical mass spectra and fragmentation patterns of derivatives of sterols and methyl esters of bile acids will be reviewed to identify fragment ions characteristic of individual compounds; chromatograms of selected fragment ions will be used to establish the nature of the parent sterol or bile acid. The use of standards labeled with stable isotopes for quantitative work will be illustrated with specific bile acid derivatives. (Supported by NIH Grant HL-07878 and the Fannie Rippel Foundation.)

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CHLORINATED SURFACTANTS. Roger Perron and Josiane Petit, Centre National de la Recherche Scientifique, 2-8 Rue Henry Dunant, 94320 Thiais, France.

Radical chlorination of fatty acids is described, and it is shown that sodium or triethanolamine salts of chlorinated fatty acids are soluble in water or in oil only for low chlorine contents. They are unstable and slowly transform into polyesters and lactones. Various substitution reactions were attempted in order to ameliorate water solubilities and emulgator properties, while an ester of a polyoxyethylene methyl ether was prepared. Limited chlorination was also applied to α-sulfonic fatty acids, and various esters and salts derivatives were obtained and studied. Superficial and interfacial tensions were measured in function of concentration for the different products, and the ability to give emulsions and microemulsions is discussed.

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CONTROLLING N-NITROSO COMPOUNDS IN COSMETICS. William J. Mergens, Hoffmann-La Roche Inc., Nutley, NJ 07110.

The use of amines or amides as ingredients in cosmetic formulations, the presence of nitrite as a contaminant, or components containing nitro groups can all lead to the formation of nitrosamines and nitrosamides, many of which are known carcinogens. The mechanisms of reactions leading to the formation of nitrosamines and nitrosamides will be discussed. These can vary considerably, depending upon the nature of the particular formulation. Means of reducing or effectively minimizing these nitrosation reactions include removal of sources of nitrosating agents, avoiding undue exposure to air, and the addition of blocking agents such as ascorbic acid, ascorbyl palmitate, or tocopherol, which operate by reduction of the nitrosating agent. These substances, thus, compete with the amine or amide in these reactions.

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A STUDY ON THE IMPROVEMENT IN THE FOAM CHARACTERISTICS OF SOAP FOAMS BY THE ADDITION OF SUPER FATTING AGENTS. Hiroji Yamada, Hideo Komatsu, and Muneo Tanaka, Shiseido Co., Ltd., 100 Tokeneke Road, Darien, CT 06820.

Super fatting agents such as fatty acids and alcohols are widely used to improve the foam characteristics of commercial soap foams. In order to study how these additives change these foam characteristics we measured viscoelasticity, bubble size of soap foams, and observed the dissolved state of soap solutions. A mixture of Na-Laurate (12% w/w), Na-Palmitate (40%), and Na-Oleate (48%) was used as a model soap throughout this experiment. Foams were generated by a pneumatic procedure introducing dry thermocontrolled air into a soap solution through fine orifices. Viscoelastic properties were measured by a device we developed, whose principle is based on the dampening effect of a foam on an oscillating coiled spring. The result found was that super fatting agents decreased the speed of gelation of the soap solution; this generated smaller bubbles. The smaller bubbles had the greater viscoelasticity due to the increase of Laplacian pressure and the increase in inter-facial area of the foam. Thus, the change in the dissolved state of the soap solutions by the super fatting agents improved the foam characteristics of the soap foams.

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PREPARATION OF SURFACTANTS BY REACTION OF FATTY ACID ESTERS WITH HYDROLYZED PROTEINS. Oliver J. Muscio, Jr., Brent Cole, Terri McCarty, and Vikas Sandu, Department of Chemistry, Murray State University, Murray, KY 42071.

A unique technique for the direct reaction of fatty acid esters with partially hydrolyzed proteins will be described. The reaction may be effected by alkaline catalysts in solvents in which both the protein and fatty acid ester components are mutally soluble. These solvents may be removed under vacuum upon completion of the reaction, and little further purification of the product appears to be necessary. The resulting fatty acid peptide condensation products are similar to those produced by reaction of the more expensive fatty acid acyl chlorides with hydrolyzed proteins showing excellent surfactancy. Their solubility in water is dependent upon the particular fatty acid ester used, as well as upon the relative proportions of protein and fatty acid. This research was supported by a grant from the Fats and Protein Research Foundation, Inc., Des Plaines, IL 60018.

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ACTION OF SURFACTANTS FOR IMPROVING THE SURFACE OF PIGMENTS. Andor Lörinc, Colouristical Review, P.O.B.182. 1390 Budapest, Hungary.

This paper presents the results which have made possible a development, by the adsorption of surfactants, of organic pigment surfaces that permits the economical production of improved dispersion paints. This required the modification of the surface properties of the pigment, instead of changing its chemical composition. Representatives of two chemical groups are discussed which, although differing in their specific function, interact to form a combination that shows better performance than the components alone. The two groups are surface active agents and organic pigments. The research started with the assumption that modern surface active agents may change the surface properties of soloids, increasing their wettability and dispersibility. The other starting principle of the investigations was the fact that solutions of surfactants have certain application values. The next task was to determine that adsorption of the ionic surfactants has really occurred by means of measuring the contact angle on the organic pigment. The results obtained with ionic surface active agents have shown that their oriented adsorption on organic pigments—on copper-phthalocyanins in our case—enhanced the wettability and thereby the dispersibility in aqueous media. This effect equally occurs either with anionic or cationic surfactants. Furthermore, it has been found that the wettability of the modified pigments, besides the morphological and chemical nature of the pigment—depends on the structure of the adsorption layer. The final result of the work established that the use of organic pigments with modified surface gave dispersion paints having improved rheological and other properties.

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A NEW ENERGY-SAVING PROCESS FOR THE PRODUCTION OF CRUDE OIL WITH EXTREMELY LOW ANISIDIN- AND PEROXIDE-NUMBERS. Thorsten Homann, Manfred Knuth, and Wolfgang Stein, c/o Fried.Krupp GmbH, Krupp Industrie—Und Stahlbau, P.O. Box 900 800, D 2100 Hamburg 90, Germany.

A new oil milling process is described, which basically consists of the well-known operations, prepressing and solvent extraction. This process differs from the normal oil milling process in that whole seeds up to approximately 10 mm particle size can be fed into a specially designed screw press without any preceding milling or heating. The crude oil leaves the press at average temperatures of 30 to 50 C, thus making it of better quality. Also, the presscake obtained shows better extractibility than that obtained from usual pressing operations. As a consequence, extraction times can be cut to achieve the same residual oil content. A comparison between the different processes regarding energy consumption and investment cost is based on experimental data from a pilot plant. The layout of production plants will be discussed.

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ENERGY SAVINGS IN EDIBLE OIL PROCESSING BY MEANS OF HEAT EXCHANGERS. Gunnar Haraldsson, Alfa-Laval AB, Sweden.

For many years, regenerative heat exchangers have been used in the edible oil processing industry in order to conserve energy. The present trend of ever increasing energy cost has, however, increased the number of applications where the installation of a regenerative heat exchanger is well justified. The concept of exergi will be briefly described, and the exergi loss during heat exchange and its relation to the temperature level will be discussed from a theoretical viewpoint. The various processes in edible oil refining where regenerative heat exchangers have been installed will be described, and examples will be given of different types of heat exchangers and how they should be dimensioned. The influence of higher vs. lower heat recovery in relation to the cost of energy and capital costs on the total process economy will be illustrated by numerical examples.

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STEAM JET PUMPS AS VACUUM PUMPS IN THE EDIBLE OIL INDUSTRY. H. Gehring, Körting Hannover AG.

Pumping of vapors and gases from distillation columns and desovessels to atmospheric pressure is done by steam jet vacuum pumps (boosters and ejectors) with intercondensers. Although overdesigning of all parts of the plants, and mainly of the vacuum pumps was formerly possible, it can no longer be maintained with respect to the demand for the lowest possible consumption of material and energy. To meet these demands it is necessary to carefully coordinate all data such as pressure of column, operating temperature, stripping steam quantity, pressure losses in the piping systems, cooling water temperature, and motive steam pressure. The paper

outlines the interdependence of these data by means of graphs and tables. The performance characteristics of ejectors and ways to save steam and cooling water are explained. Flow sheets and photographs of existing plants are shown and measures to reduce waste-water load are mentioned. At the conclusion of the paper, alternatives to the above-described vacuum systems are given together with a comparison of operating costs of various pump systems.

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UTILIZATION OF ZEARALENONE-CONTAMINATED CORN FOR ETHANOL PRODUCTION. G.A. Bennett, A.A. Lagoda, O.L. Shotwell, and C.W. Hesseltine, Northern Regional Research Center, 1815 N. University, Peoria, IL 61604.

Two lots of yellow corn, severely damaged by *Fusarium* fungi and contaminated with 8.0 and 33 ppm zearalenone, respectively, were used for ethanol production. A laboratory procedure similar to that used by the fermentation industry was employed to produce stillages that were 7.2 to 9.0% ethanol. Ethanol was recovered by distillation, and the residual grain solids by filtration and drying. No toxin was detected in the ethanol fraction. Zearalenone was not destroyed by the fermentation process but was concentrated in the residual grain solids and solubles, which are generally used as animal feed. Decontamination of residual solids was accomplished by treating the wet solids with formaldehyde or ammonium hydroxide prior to drying at 50 C.

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AN IMPROVED METHOD FOR THE PURIFICATION OF GOSSYPOL ACETIC ACID. S.I. Zhou, D.H. Liu* and L.Y. Zhong, Wuxi Institute of Light Industries, Wuxi, Kiangsu, People's Republic of China.

Gossypol isolated from cottonseed flakes was converted to dianilinogossypol, which was hydrolyzed in aqueous acetic acid by means of concentrated sulfuric acid, and distilled water was added. Gossypol acetic acid was precipitated on standing, and further hydrolyzed in acetone with concentrated sulfuric acid to remove unhydrolyzed dianilinegossypol. Crude gossypol acetic acid was dissolved in acetone. Recrystallization of gossypol acetic acid was performed by the addition of glacial acetic acid, and successive recrystallization was carried out once or twice in the same manner. Pure gossypol acetic acid was obtained, with an m.p. of 176–178 C., ash content of 0.02 to 0.07% and purity of 98.0 to 102.0%. The yield of pure gossypol acetic acid was about 45%, based on the amount of dianilinogossypol hydrolyzed. No ethyl was used in this process. This preparation meets the requirement of oral contraceptive trial.

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LIGHT INDUCED INTERACTIONS OF CARBOXYLIC ACIDS WITH POLYCYCLIC AROMATIC HYDROCARBONS. M.K. Logani, W.A. Austin, and R.E. Davies, Temple University School of Medicine, The Center for Photobiology, 3322 North Broad Street, Philadelphia, PA 19140.

The hypothesis that carcinogenic activity of polycyclic aromatic hydrocarbons in the presence of light should be increased has given several conflicting results when tested in vivo. Different results such as acceleration, inhibition, or no change in the carcinogenic activity have been reported. In our attempt to understand the nature of variables responsible for these conflicting results, we have studied the in vitro photoinduced interactions of polycyclic aromatic hydrocarbons with carboxylic acids. These acids were chosen because fatty acids constitute about 30% of human skin surface lipids. Our previous studies have shown that benz[a]pyrene reacts with fatty acids very efficiently when a mixture of two components is irradiated with xenon arc lamp in solution in a pyrex container. The predominant product, particularly in nonpolar solvents, was characterized as 6-acyloxybenz[a]pyrene by means of UV, IR, NMR, and mass spectroscopy. In the present studies, we have investigated the photoinduced interaction of benz[a]pyrene and other polycyclic aromatic hydrocarbons with saturated carboxylic acids in the presence of some unsaturated lipids. In the case of benz[a]pyrene, it is shown that: (a) the presence of unsaturated fatty acids or their esters markedly inhibit the photoinduced acyloxylation of BP by saturated acids–squalene and cholesterol benzoate show a similar effect; (b) total consumption of BP is not affected significantly, indicating the involvement of competing reactions with unsaturated molecules; (c) the effect is concentration dependent; and (d) the inhibition is not affected by the presence or absence of oxygen. It is inferred from these studies that rapid destruction of polycyclic aromatic hydrocarbons in the presence of light and carboxylic acids involves other pathways besides photoacyloxylation. Whether a direct or indirect participation of carboxylic acid is involved in these other pathways remains to be understood. Significance of these photochemical reactions in photobiological studies will be discussed.

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THE EFFECT OF FREEZER STORAGE ON THE AUTOXIDATION OF FISH LIPID FRACTIONS. Ludmila Stodolnik, Academy of Agriculture, Institute of Marine Technology, Kazimierza Krolewicza 3, 71-550 Szczecin, Poland.

Studies were carried out on oxidation of lipid constituents of Baltic cod and herring muscle tissues which had been freezer-stored at -30 C. Oxidation rate and stage were determined for the following lipid fractions: non-esterified fatty acids, triacylglycerols, phospholipids, and cholesterol esters. Results of these studies indicated that the particular lipid fractions in both species of fish are not equally susceptable to oxidation. Free fatty acids of herring muscle undergo the most rapid and extensive oxidation, whereas oxidation of triacylglycerols takes place at a slower rate. Phospholipids are oxidized much less rapidly, and the cholesterol esters are most resistant. In contrast, in cod muscle, the triacylglycerols undergo the most extensive oxidation, followed by free fatty acids, phospholipids, and cholesterol esters, in that order. In addition, the degree of oxidation of the particular lipid fractions of cod is much greater than that of the Baltic herring fractions. In both species the extent of oxidation of each specific lipid class depends not only on the proportion in the total lipid but also on the total lipid content of the fish muscle.

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LIPID COMPOSITION OF STOMACH OILS AND SUBCUTANEOUS FAT OF MUTTON BIRDS *(PUFFINUS TENUIOSTRUS;* ORDER PROCELLARIIFORMES). David G. Bishop, Janette R. Kenrick and June Olley, Plant Physiology Unit, CSIRO Division of Food Research, P.O. Box 52, North Ryde 2113, Australia; Graham W. Hosie and David A. Ritz, University of Tasmania.

The procellariiform birds are characterized by their ability to store quantities of oil in the proventriculus of their alimentary tracts. This oil is presumed to be of dietary origin. We have measured the content and composition of this oil from adult mutton birds and from chicks, who during the first months of their lives are fed exclusively on food regurgitated from the adult bird, and compared it with that of the subcutaneous fat. The average oil content of the stomach of adult birds (approx. 1 g/100 ml) is low compared to that of chicks (approx. 45 g/100 ml). Analysis of the oil of adult birds showed the presence of substantial amounts of wax esters and triglycerides. The subcutaneous fat consisted exclusively of triglycerides whose fatty acid composition did not reflect that of the stomach oil, because the fat had a substantially higher content of octadecenoic acid and lower content of docosahexaenoic acid than did the oil. The oil content of chick stomachs also consisted predominantly of wax esters and triglycerides. The fatty acid composition of this oil however, differed from that of adult oil, especially in having a higher content of octadecenoic acid. Chick subcutaneous fat consisted exclusively of triglyceride, and its fatty acid composition was similar to that of the stomach oil. As the stomach oil of chicks would have been derived from food already subjected to some degree of digestion and absorption in the stomach of the parent, some preferential hydrolysis of those lipids containing polyunsaturated fatty acids, in the parent stomach, is indicated.

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N-6 FATTY ACIDS IN WILD AND CAPTIVE DOLPHINS. G. Williams and M.S. Crawford, Department of Biochemistry, Zoological Society of London, London N.W.1., United Kingdon; D.C. Malines, Northwest and Alaska Fisheries Center, Seattle, WA.

Lipids of land mammals contain proportions of n-3/n-6 acids of between 0.3/1 and 0.2/1; lipids of marine vertebrates contain proportions of up to 50/1. Hence, one might expect marine mammals to exhibit a n-3/n-6 ratio, reflecting the relatively high n-3 content of the food. When two Bottlenose Dolphins *(Tursiops truncatus)* died at London Zoo, the fatty acid ratios in the lipids were between 3/1 and 1/1, which is closer to land mammals and much higher than their fish diets. In addition, muscle, liver, and brain phospholipids obtained from wild dolphins contained arachidonate (AA) levels three times those found in the captive dolphins. Wild dolphins may select food rich in AA and/or may have an especially efficient concentration mechanism for n-6 fatty acids. The low level of AA in the captives suggests that they were unable to bioconcentrate sufficient amounts from the fish fed to them. A richer source of AA may exist for wild dolphins to reflect such high quantities in their tissues. On the basis of this limited data, it appears that food sources richer in AA than most cold water fish may be needed to provide adequate nutrition for captive dolphins. Dolphins may require significant amounts of the "essential" n-6 acids to maintain the integrity of biomembranes. Also, the dolphin, being a placental mammal, may well require AA as a prostaglandin precursor needed, for example, in reproduction.

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A BRIEF EVALUATION OF THE SAFETY OF PARTIALLY HYDROGENATED MARINE OILS (PHMO) IN THE HUMAN

DIET. 1. CARDIAC LIPIDOSIS PHENOMENON IN EXPERIMENTAL ANIMALS. Stuart M. Barlow and Iain F. Duthie, International Association of Fish Meal Manufacturers, Hoval House, Orchard Parade, Mutton Lane, Potters Bar, Herts, EN6 3AR, England.

Limited expermental work, published early in this decade, indicated that if weanling rats are fed high levels of certain C22:1 containing PHMO, a cardiac lipidosis phenomenon could be demonstrated, i.e., intracellular lipid droplets measured by chemical and/or histological means. Subsequent information, particularly some which was recently published, assists assessment of the significance of the phenomenon. It is remarkable that the effect regresses rapidly, even when the feeding regimen is maintained. The effect is less pronounced in older rats and in weanling pigs, suggesting age and species differences. Rat heart mitochondria metabolize C22 monoenes, at different rates. Species differences in mitochondrial metabolism have been demonstrated. Other subcellular metabolic mechanisms may come into play. Unhydrogenated marine oils of equivalent type give a lipidosis effect in rats, showing that PHMO do not differ qualitatively from them in this respect. Lipidosis also occurs in animals fed control fats. The effect can even be induced in rats and mice by merely withdrawing oridinary food for short periods. A low grade lipidosis, not associated with C22:1 consumption, could be the usual human cardiac state. C22 monoenes are found in control animal heart lipids, and in those of humans not thought to consume C22:1-containing fats. Further research in such areas would be helpful in increasing understanding of this many faceted subject. It can be suggested at this stage, however, that cardiac lipidosis is not an unphysiological event, that C22 monoenes are not foreign to the mammalian organism, and that there are metabolic mechanisms, possibly adaptive, capable of dealing with both aspects. Various types of PHMO have been eaten by people for the past 60 years, yet study of the literature does not reveal any specific link between human ailments, such as coronary and ischaemic heart diseases, and PHMO consumption. Greenland Eskimos, with marine C22:1 present in serum triglycerides, are noted for low susceptibility to cardiovascular disorders.

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A BRIEF EVALUATION OF THE SAFETY OF PARTIALLY HYDROGENATED MARINE OILS (PHMO) IN HUMAN DIET WITH REGARD TO: 2. The longer term cardiac lesion phenomenon in experimental animals. I.F. Duthie, "Shawnee", Twinoaks, Cobham, Surrey, KT11 2QW; S.M. Barlow, IAFMM, Hoval House, Orchard Parade, Mutton Lane, Potters Bar, Herts, EN6 3AR, England.

Very limited work published in the early 1970s suggested that feeding PHMO to young male rats might produce the same phenomenon observed when rapeseed oils were fed at high levels and for moderately long periods to rats, namely, myocardial lesions generally classified as microscopic focal necrosis and/or fibrosis, Canadian PH herring oil very high in C22 monoenes was used, which is not representative of world production and consumption of marine oils, and the experimental results were unsupported by descriptions of histological findings, particularly those from the control rats. Subsequently more extensive work demonstrated that feeding rats pH capelin oil (Norwegian), typical of much fish oil in world trade, and also PH herring oil (Canadian), was not associated with lesion effects that were different from those associated with the negative control lipid. Such lesions have not been demonstrated in hearts of pigs fed control lipid or PHMO-containing diets. Hearts of monkeys given either high-level control lipid or PH herring oil-containing diets for extended periods showed no differences in lesion effects. Many feeding studies on rapeseed oils have been published providing conflicting information. Two points are clear, however, Firstly, these studies amply confirm that the lesions occur in control rats; thus, the implication from the early papers that high erucic acid rapeseed (HEAR) oils produce a unique cardiac lesion, would seem to be invalid. Secondly, the low erucic acid rapeseed (LEAR) oils are still associated with lesions to a greater extent than control and not much less than HEAR oils, considering the near elimination of erucic acid from some of them. This casts serious doubts on the contention that erucic acid, *per se* or partly, and docosenoic acids generally, are uniquely responsible for the cardiac lesions. It would seem that to the extent that rapeseed oils produce a cardiac effect in the rat, it may be exacerbation of an existing or intercurrent condition. There is no agreement as to what factors in HEAR and LEAR oils might be responsible. In any event, attributing rapeseed oil effects to the PHMO family of products is not justified by the evidence available.

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APPLICATIONS OF THE MINI-COMPUTER IN MARINE FATTY ACID ANALYSIS. Jeanne D. Joseph, Charleston Laboratory, P.O. Box 12607, Charleston, SC 29412.

A mini-computer with a memory of 16K bytes can be used very effectively in both qualitative and quantitative GLC analyses of fatty acid methyl esters (FAME). Relative retention times and equivalent chain length values can be calculated far more rapidly with a mini-computer than with calculators commonly available in most laboratories, facilitating fatty acid identifications by graphic or arithmetic methods. The mini-computer can be used even more effectively in applying the detector and column correction factors that are often required for accurate quantitation of GLC analyses carried out on stainless steel capillary columns. The output of the quantitation program produces the results as both weight percent and mole percent compositions and, in addition, provides a calculated iodine value and the average molecular weight of the FAME sample. These calculations would be laborious on programmable calculators which may cost as much as, if not more than, many available mini-computers. The programs have been written in BASIC language on a computer which accepts alpha-numeric characters, and are interactive, which makes them very simple to use. The application of these programs in a typical FAME analysis will be illustrated.

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STEREOSELECTIVE CONTROL OF THE WITTING REACTION: PREPARATION OF METHYL *cis,cis-*, *cis,trans-*, *trans,cis-* and *trans,trans*-12,15-OCTADECADIENOATE–9,10-d_2. Henry Rakoff and Edward A. Emken, Northern Regional Research Center, AR, SEA, USDA, 1815 North University, Peoria, IL 61604.

The four geometric isomers of methyl 12,15-octadecadienoate-9,10-d_2 were prepared by the Witting reaction between *cis-* or *trans*-3-hexenyltriphenylphosphonium bromide and methyl 12-oxododecanoate-9,10-d_2 in ethyl ether with butyl lithium as the base. The distribution of isomers formed in the Witting reaction can be controlled to give predominantly either *cis* or *trans* geometry. Reaction at room temperature gives about 85 to 90% *cis*. Reaction at –40, followed by equilibration of the unstable Witting intermediate with methanol at that temperature, gives a product mixture containing about 60% *trans* geometry in the double bond generated. The mixtures of *cis,cis* and *trans,cis* and of *cis,trans*, and *trans,trans* isomers were separated by silver resin chromatography, and the pure individual isomers were converted to their corresponding triglycerides.

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SYNTHESIS OF α-METHYLENE SUBSTITUTED FATTY ACIDS. S. Serota and W.M. Linfield,* Eastern Regional Research Center, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, PA 19118.

A facile, four-step procedure was developed for the synthesis of α-methylene substituted fatty acids in an overall yield of about 75%. In the first step, the fatty acid was condensed with 2-amino-2-methylpropanol (AMP) to form the amide, which upon heating in vacuo at 180 C yielded the corresponding oxazoline in an 80 to 85% yield, the remainder being the fatty ester amide of AMP. The oxazoline reacted readily with paraformaldehyde at 90 to 100 C to give an intermediate mixture of mono- and bis-methylol derivatives, which upon heating for 2 hours in refluxing xylene yielded about 70% of the oxazoline derivative of the structure $RC(=CH_2)-C=N-\overbrace{C(CH_3)_2-CH_2-O}$. Repeated treatments with paraformaldehyde and heating in refluxing xylene gave a 95 to 97% overall conversion to the desired substituted oxazoline. Subsequent acid hydrolysis of this oxazoline, followed by saponification with alkali and acidification of the resulting alkali metal salt, gave an essentially quantitative yield of the desired α-methylene substituted fatty acid of a 95% or higher degree of purity of the crude product, as determined by GLC and neutral equivalent of the product. The α-methylene derivatives of lauric, myristic, palmitic, stearic, and oleic acids were prepared in this manner. Unlike paraformaldehyde, higher molecular weight aliphatic aldehydes and benzaldehyde did not react with the fatty oxazolines. The imidazoline prepared via the reaction between a fatty acid and N-(2-hydroxyethyl)-1,2-ethylene diamine did not react with paraformaldehyde under the conditions described above.

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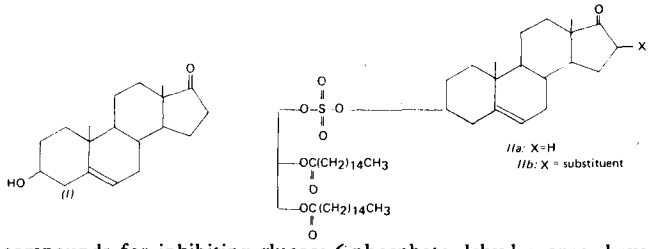
IMPROVED SYNTHESIS OF THE BIOLOGICALLY ACTIVE SULFOLIPID, SULFATIDYL-DEHYDROEPIANDROSTERONE, AND RELATED COMPOUNDS. Magid A. Abou-Gharbia, Laura Pashko, Arthur G. Schwartz, and Daniel Swern, Fels Research Institute and Department of Chemistry, Temple University, Philadelphia, PA 19122.

Dehydroepiandrosterone (DHEA) (*I*), an adrenal steroid found in subnormal plasma concentrations in women predisposed to develop breast cancer, inhibits the formation of spontaneous mammary cancer in female C3H(A^{VY}/a) mice, as well as reducing their weight gain. *I* also protects cultured rodent cells against dimethylbenzanthracene(DMBA)- and aflatoxin B_1- induced cytotoxicity

and transformation, and inhibits the metabolism of (^{3}H)DMBA to water soluble products. *I* is a potent noncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase, the enzyme responsible for generating the bulk of extra mitochondrial NADPH. Chemical carcinogens such as DMBA and aflatoxin B_1 require metabolic activation by NADPH-requiring mixed function oxidases. We have postulated that *I* protects cultured cells against the toxic and transforming effects of these carcinogens by inhibiting their activation as a result of lowering of NADPH levels. Since the dosage of *I* is quite high in control of breast cancer, we are seeking analogs of considerably higher potency to minimize or avoid undesirable side effects of high-dosage steroid therapy. Two promising types of



compounds for inhibiting glucose-6-phosphate dehydrogenase have been synthesized, sulfatidyl dehydroepiandrosterone(*IIa*) and substituted derivatives(*IIb*). Synthetic paths will be discussed, as well as the importance and significance of the sulfolipid moiety. Cancer prevention studies will be presented elsewhere.

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CHEMICAL AND BIOLOGICAL PROPERTIES OF UNUSUAL PHOSPHOLIPIDS. W.J. Baumann, S. Parthasarathy, Y. Wedmid, and R. Murari,* The Hormel Institute, University of Minnesota, 801-16th Avenue N.E., Austin, MA 55912.

Procedures were developed for the chemical synthesis of phospholipids varying in their constituent base, polyol backbone, and aliphatic functions. Routes and conditions were chosen that would minimize by-product formation and be versatile enough to synthesize choline and ethanolamine phospholipids containing glycerol or short-chain diol backbones, and bearing long-chain acyl, alkyl, and alk-1-enyl moieties. Carbon-13 nuclear magnetic resonance spectrometry, in particular, was utilized to define the structures of intermediates and final products of phospholipid synthesis. It was shown that phospholipid aggregation can be minimized in chloroform-methanol-water, 50:50:15 (by volume), and that distinct carbon-13 chemical shifts and precise couplings of phospholipids in solution can be measured. Two- and three-bond C-P couplings as well as C-N couplings provided information on the conformational arrangement of polyol backbones and polar head groups of phospholipids in solution and facilitated chemical shift assignments. Carbon-13 NMR spectra of synthetic phospholipids aided in the assignment of carbon signals in the spectra of native biological and reconstituted phospholipid compelexes including myelin, lipoproteins, platelets, microsomes, and others. Membrane modification by various lysolecithin-type monochain phospholipids is illustrated for red blood cells, tumor cells, and myelin.

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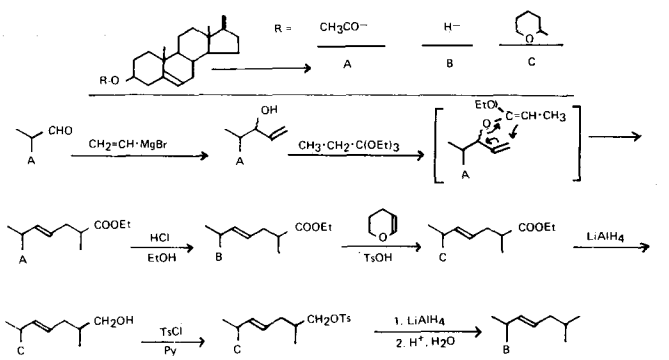
A NEW SYNTHESIS OF 22-DEHYDROCHOLESTEROL. Henry W. Kircher and Fumiko U. Rosenstein, Department of Nutrition and Food Science, University of Arizona, Tucson, AZ 85721.

A synthesis of *trans*-22-dehydrocholesterol based on the oxy-Cope rearrangement was devised:



The product was identical to that prepared from the aldehyde with triphenyl 3-methyl-butyl phosphorane and separation of *cis-trans* Δ^{22} isomers (*Lipids, 12,* 297 [1977]), and that prepared last year (*JAOCS, 55,* 236A [1978]) by a less convenient synthesis with a ketene N, O-acetal.

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TRANS-CIS ISOMERIZATION OF UNSATURATED FATTY ACIDS. M.S.F. Lie Ken Jie, Chemistry Department, University of Hong Kong, Hong Kong.

Cis-trans isomerization of unsaturated fatty acids can be readily achieved by various methods, but the reverse process, *trans-cis,* is a more difficult process thermodynamically. The halogenation-dehalogenation process was used to transform *trans*-isomers to the *cis*-isomers in ca. ninety percent yields with no double-bond migration in mono- and diunsaturated fatty acids, provided the double bonds in the latter are not conjugated. The results of the conjugated unsaturated fatty acids will be discussed in detail.

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ENVIRONMENTAL CONCERNS IN INDUSTRY. James J. Bolger, Environmental Research Group, 7303 W. 90th Street, Bridgeview, IL 60455.

The primary concerns of industry in dealing with the environment have and will continue to be: (A) the economic impact of control systems on profits, operations, and product marketability; and (B) the availability of adequate engineering technology for resolving the present and future regulatory requirements. This presentation will be an outline of the four most significant laws which have closed the environmental loop around manufacturing facilities: Clean Air, Clean Water, RCRA, and TSCA. An accounting of the investment and operating expense dollars which have been spent over the last decade will be given, with the vegetable oil industry highlighted. Anticipated expenditures by both government and industry will be discussed, with mention of the posture taken by President Carter and the EPA on future programs. Finally, an outline of the "state of the art" in pollution control technology, specifically designed to remedy-refiner and processor problems, will be provided. This outline will be "technology," rather than "manufacturer" slanted, and will deal with available systems.

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RESOURCE CONSERVATION AND RECOVERY ACT (RCRA) IMPACTS ON INDUSTRY. Michael J. Boyer, The Chester Engineers, 296 Interstate North, Suite 110, Atlanta, GA 30339.

In 1976 the Federal Government passed the Resource Conservation and Recovery Act (RCRA). While the final regulations dealing with the implementation of the Act have been long in coming and are still in draft form at this time, RCRA will undoubtedly have a significant impact on industry environmental programs. This paper examines RCRA, particularly with regard to ultimate handling and disposal of those items which it covers that are relevant to the edible oil refining, fatty acid processing, and related industries. At the time when this abstract was prepared, regulations had not been finalized. The paper and presentation will address final regulations, if they have been promulgated, or anticipated regulations if they are still in draft form.

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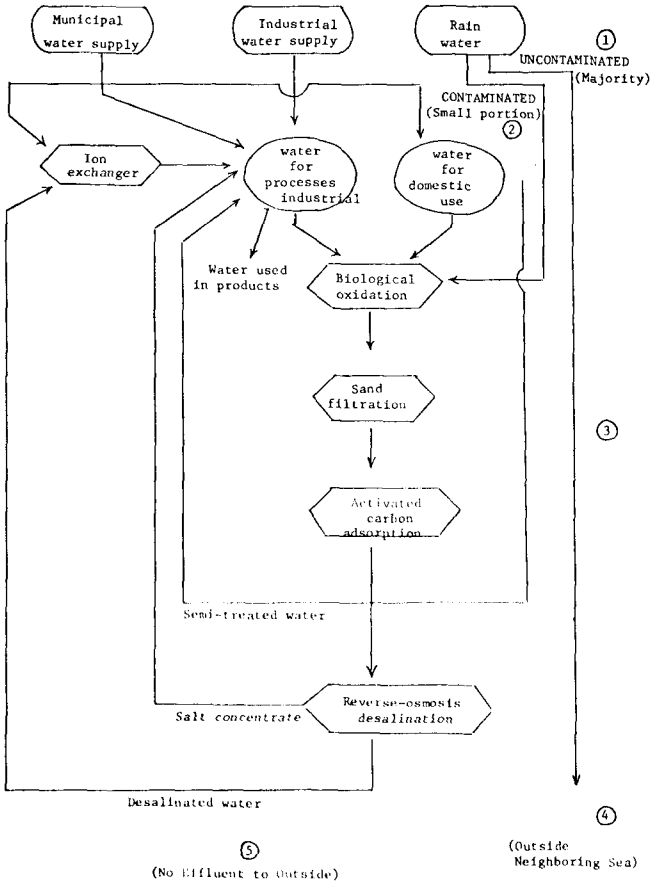
THE COMPLETELY CLOSED WATER USAGE SYSTEM IN A DETERGENT PLANT. T. Kinoshita and M. Tanigaki, Masuo Sugai, Assistant to the President, Kao Soap Company, Ltd., 1, Kayabacho, Nihonbashi, Chuo-ku, Tokyo 103, Japan.

Anti-pollution regulations and water shortages are big problems in Japan, too. To cope with these problems, the realization of a completely closed system based on free recycling of the water will be the best solution. The important factors are to minimize water requirements and to effectively use treated water, taking into consideration the different qualities of the water required. Kao Soap completed such a system, one which does not leak out any contaminated water from its Kyushu plant. The plant is located on the seashore of the Japanese Mediterranean Sea. It produces 10,000 to 15,000 tons of various detergents per month. More than 10,000 tons of water is used each month by the inhabitants for production, cooling, cleaning, and the personal needs of daily living. The treatment process consist of biological oxidation, sand filtration, activated carbon adsorption, and reverse-osmosis desalination. A flow diagram of the system is below. By the completion and operation of the system, we are able to save about one-third of the water without risking the contamination of the inland sea.

Continued on next page.

Abstract No. 297.



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TREATMENT OF A TALL OIL EFFLUENT WITH INDUCED AIR FLOTATION FOLLOWED BY BIOLOGICAL OXIDATION. J.P. Krumbein, Director of Engineering, Reichhold Chemicals, Inc., P.O. Box 1433, Pensacola, FL 32596.

Treatment of a tall oil effluent with induced air flotation followed by biological oxidation effectively removes conventional pollutants. BOD, COD, and oil and grease removals exceeding 95%, 85%, and 98%, respectively, are obtained. Other advantages include comparatively low capital investment and operating costs as well as good flexibility of operations when compared with other systems achieving comparable effluent improvement. Two tall oil distillation plants are now successfully employing this process.

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ENVIRONMENTAL PROTECTION IN PHYSICAL REFINING OF OILS AND FATTY ACIDS DISTILLATION. Hermann Stage, Destillationstechnik, Emdenerstr. 10, 5000 Koln 60, Germany

The pollution of air and water caused by installations now mainly used for physical refining of oils,for straight-run distillation, and for fractionation of fatty acids, is too high according to today's laws in industrial countries. We found that the pollution is a function of the distillation conditions and especially of the process sequence, the heating and condensation modes, the open steam consumption in relation to the working pressure, and last but not least, the bottom temperatures. By changing these parameters, it is possible to influence the pollution intensity. On this basis we developed economically workable processes for physical refining as well as for fatty acid distillation that caused nearly zero pollution of air and water. Another result of our process conditions—the lowest possible running costs—depends on the drastic reduction of the open steam consumption in the main distillation steps.

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PROCESS FOR RECOVERING MARKETABLE PRODUCTS FROM WASTE WATER SLUDGES. Charles Greenfield, Dehydro-Tech. Corporation, #6 Great Meadow Lane, E. Hanover, NJ 07936.

The recovery of dry solids and oils or fats from waste water-sludges with 1% and higher solids content is made possible by the use of multi-effect evaporation to dryness and the use of an oil fluidizing system. This technique, known as the Carver-Greenfield Process, is applied to recover oils or fats and protein solids as marketable products with low energy use. Examples are given for some existing installations along with pilot plant studies for some proposed plant waste sludges.

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EVALUATION OF CULTURED BACTERIA AS A BIOMASS FOR ACTIVATED SLUDGE TREATMENT OF WASTES FROM AN EDIBLE OIL REFINERY. Thomas J. Walsh, A. Panno and John Keigher, SCM Corporation, and Durkee Foods, Div. SCM Industrial Foods Group, Joliet Plant, Joliet, Illinois.

Plant scale tests from January 1978 through May 1979 covering three periods with indigenous bacteria and 4 periods with cultured bacteria were analyzed statistically using plant operating variables as standards. Performance with cultured bacteria was less subject to variation in feed conditions, recovery was more rapid after an upset and plant waste load removal efficiency was significantly improved. Data for the entire period under discussion are reviewed using COD as a measure of waste load. Statistical evaluation of data is necessary due to variability of individual points. We are using cultured bacteria to insure optimum performance of the waste treatment facility.

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WASTEWATER TREATMENT FOR EDIBLE OIL REFINERIES. Wendelin C. Seng, Globe Engineering Co., 222 No. Dearborn Street, Chicago, IL 60601.

Systems and practices for successful treatment of edible oil refinery wastewater are reviewed. Effective wastewater treatment depends upon total plant good housekeeping, conservation of water usage, integrated treatment system design, accurate control and close supervised operation. The amount of wastewater and contaminants can often be reduced up to 50 percent simply by instituting better housekeeping and water conservation procedures. Integrated treatment system design requires precise and complete definition of: (a) final effluent quality requirements (local, state and federal) and corresponding governmental charges; (b) determination of in-plant wastewater source rates, contaminant levels, pH, around-the-clock and corresponding treatability data; (c) installation of a system capable of doing the total job; (d) economic and practical recovery disposal of by-products such as sludge skimmings. Dissolved air flotation and biological treatment are examples of effective processes for water purification. The by-products are less easily recovered/disposed using these techniques, however, an industry-wide program is urged for wastewater treatment process information exchange, development and disposal.

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ELECTRO-COAGULATION FOR REMOVAL AND RECOVERY OF POLLUTANTS FROM MEAT PACKING HOUSE LIQUID EFFLUENT. O.A. Clemens, Dravo Corporation, Neville Island, Pittsburgh, PA 15225.

A new system is in use at a meat packing house for removal and recovery of pollutants. The System employs electrocoagulation and operates at the isoelective point of the pollutants, which is achieved using sulphuric acid. Results have shown: municipal environmental standards are met; low operating cost for chemicals; very low volume of skimmings; high solids content in skimmings; low rendering costs and high quality tallow. Economics indicate systems can pay for operating costs and under certain conditions amortize capital investment.

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AGE DEPENDENT FORMATION OF OXYGEN RADICALS, LIPID PEROXIDATION AND CHANGES IN BIOLOGICAL MEMBRANE FUNCTION. D. Hegner and H. Nohl, Institut für Pharmakologie, Toxikologie und Pharmazie Fachbereich Tiermedizin d. Universität München, Königinstr. 16 D-8 München.

The liquid crystalline fluid state of biological membrane is an essential condition for maintenance of different membrane functions. The liquid crystalline state of rat heart mitochondria, human erythrocytes and rat liver plasma membranes charges with the age of the organism. The degree of unsaturated fatty acids decreases and the content of cholesterol increases during aging. It could be shown that superoxide-radicals originate from minor side reactions of oxidoreductase enzymes. Aging increased the amount of superoxide radicals. A small amount of O_2-radicals escape quenching by superoxide dismutase and react with unsaturated fatty acids of the membrane in vivo. The formation of O_2-radicals is attributed to degradation of membrane lipids. The age-dependent changes in membrane lipid composition influence respiratory activity, respiratory control, P:O ratios and ATP–ADP translocase activity in mitochondria of old rats. Rat liver plasma membrane lipids also show that lipid peroxidation during aging decreases membrane fluidity and results in a change of transport parameters for cholic acid and thymidine. The change of age-dependent lipid-protein interactions was demonstrated by spin-label measurements in model membranes. The results demonstrate that peroxidative breakdown of lipids is an ongoing post-transcriptional process of aging. The

possible role of protective repair mechanisms is discussed.

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BIOSYNTHESIS OF CHOLESTEROL FROM [1-^{14}C] OLEIC, LINOLEIC AND ARACHIDONIC ACIDS. Ménouer Bouchene, Danielle Lapous and Jeanine Raulin,* Laboratoire Nutrition Cellulaire & Lipophysiologie (C.N.R.S.), Université Paris 7, 2 place Jussieu, 75221 Paris Cedex 05, France.

This investigation compared acetyl CoA esters from essential fatty acid (EFA) degradation to non-EFA degradation esters in further cholesterol synthesis in vivo. Suckling rats were kept 6 days with their mother while receiving a stock diet with 4% lipids. The young rats were then injected with 10 μCi[1-^{14}C] oleic, linoleic or arachidonic acid. Lipids were obtained from the livers: (a) by extraction with chloroform-methanol-water, (2) by treatment (2 hr, 120 C) with 1.2N NaOH and acidification, or with 6N H_2SO_4. Lipids in benzene were separated by TLC. Free cholesterol was purified by TLC as well as esterified cholesterol after 2 hr 100 C treatment with 2N NaOH-methanol. Cholesterol determination was performed by GLC using glass capillary columns and radioactivity was measured on TMS derivative. Cholesterol specific radioactivity (SRA) was found higher in livers treated with acid and alkali than in those extracted with solvents, suggesting that the newly formed cholesterol was better released when using hydrolytic procedures (especially acid hydrolysis). The data were in good agreement with our previous results from experiments with rats born to females kept 11 days on fat free diet. Cholesterol SRA was found significantly different between the experimental groups, and SRA was higher when animals were injected with labeled linoleic and arachidonic acids than when they received oleic acid for tracer. The results for cholesterol SRA when rats were injected with arachidonic acid were higher than when linoleic acid was infected. The bulk of non-labeled fatty acids was: oleic acid 10%, linoleic acid 17% and arachidonic acid 13% in livers of these suckling rats. EFA, abundant and bound to enzymes, could be very much involved in the rate of in vivo cholesterol production.

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THE MECHANISM AND CONSEQUENCES OF LIPID PEROXIDATION IN ISOLATED HEPATOCYTES AND *IN VIVO*. Johan Högberg, Irene Anundi and Jovan Rajs, Department of Forensic Medicine, Karolinska Institutet, S-104 01 Stockholm 60, Sweden.

During the last decade studies on the univalent reduction and activation of oxygen have implicated glutathione (GSH) peroxidase in animal tissues. It can be assumed that an inactivation of one or both of these enzymes will lead to increased production of the hydroxyl radical or other toxic products of oxygen activation in the respiring cell. We tested the hypothesis that lipid peroxidation can be induced by GSH deficiency and found that GSH-deficient hepatocytes accumulate malondialdehyde (MDA). Isolated rat hepatocytes were depleted of their GSH by electrophiles such as chloro- or iodoacetamide. When incubated in an amino-acid free medium, these cells could be kept in a state of GSH-deficiency for several hours, provided α-tocopherol was included in the medium. When α-tocopherol was excluded, MDA started to accumulate after a time lag of about one hour. Cysteine or methionine (precursors for GSH synthesis) prevented MDA accumulation. Cells which accumulated MDA eventually lysed. In nonstarved rats injected i.p. with the electrophile there was a rapid drop in hepatic GSH levels and a complete recovery was not seen for 48 hours. These animals exhibited enhanced hepatic MDA levels as long as the GSH level was decreased and their livers showed hydropic degeneration in the peripheral 2/3 of the lobule. In starved rats midzonal necrosis developed within 6-8 hours. These studies show that lipid peroxidation can be induced in rat liver cells by GSH deficiency. The in vivo studies indicate that lipid peroxidation can be significantly increased without causing permanent damage to hepatocytes in the peripheral parts of the lobule.

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EFFECT OF FREE FATTY ACIDS ON SUBENDOCARDIAL PURKINJE FIBERS IN THE CANINE HEART. John J. Fenoglio, Jr., M.D., Department of Pathology, 630 West 168th St., New York, N.Y. 10032.

The purpose of this study was to determine whether the increased level of circulating free fatty acids present following myocardial infarction depressed Purkinje fiber action potentials. A myocardial infarct can be reproduced in dogs by a two-stage ligation of the left anterior descending coronary artery. The subendocardial Purkinje fibers survive over the infarcted area of the left ventricle. Twenty-four hours after occlusion, the transmembrane potentials are altered and spontaneous depolarizations are recorded. These fibers are the site of origin of the ventricular arrhythmias observed at this time. Structurally, the Purkinje fibers are intact but contain increased numbers of lipid droplets suggesting that changes in lipid metabolism are responsible for the arrhythmias. Isolated preparations of left ventricular myocardium excised from normal dogs were superfused in Tyrode's solution containing high concentrations of commercially available free fatty acids. Transmembrane potentials were recorded from the subendocardial Purkinje fibers over a ten hour period. The transmembrane potentials were altered (action potential duration prolonged and V_{max} depressed), however, spontaneous depolarizations were not seen. There was no increase in lipid droplets in the Purkinje fibers with altered transmembrane potentials. Free fatty acids do alter the transmembrane potentials of Purkinje fibers but are not related to the accumulation of lipid by these cells nor to the ventricular arrhythmias arising from the subendocardial Purkinje fibers 24 hours after myocardial infarction.

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MODULATION OF MEMBRANE PROTEINS BY LIPID FLUIDITY AND AGING. Meir Shinitzky, Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel.

The aging process in both mouse and man is associated with a progressive change in lipid composition of cell membranes. The most prominent changes are: increase in the ratio of free cholesterol to phospholipids, and decrease in the ratio of lecithin to sphingomyelin. These changes elevate the lipid microviscosity to a similar extent by different modes of action, and consequently affect the dynamics of the membrane proteins and modulates their degree of exposure to the external surrounding. The latter type of lipid-protein interplay is termed "passive modulation" and probably plays an important role in regulating the expression of membrane antigens and receptors. Autoimmune diseases and the overt reduction of responses to hormones, which occur with aging, may be related to passive modulation of antigens and receptors.

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FUNGAL PHOSPHOLIPIDS DURING AGING AND GROWTH WITH α-TOCOPHEROL. Satruken Ramsammy and R. Cecil Jack, Department of Biological Sciences, St. John's University, Jamaica, NY 11439.

In this paper, we report on the fatty acid composition of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from cultures of the fungus *Glomerella cingulata* grown with or without supplements of α-tocopherol. Previously, we reported on a shift of fatty acid composition in triacylglycerols and total phospholipids from cultures of *G. cingulata* treated with α-tocopherol and examined the effects of α-tocopherol on the labeling and turnover of *G.cingulata* lipids. PC and PE were isolated from mycelial samples of α-tocopherol treated *G. cingulata* and control cultures at 48, 60, 72, 96 and 120 hours of growth; fatty acid methyl esters were then prepared and analyzed by standard procedures. We found α-tocopherol increased the ratio of unsaturated/saturated fatty acids (U/S) in PC of the treated cultures as much as 200%, but changed the number of double bonds/mole (Δ/mol) only $\pm$ 6%. The corresponding changes in PE were a maximum increase in U/S of 30% and a change in Δ/mol of approximately $\pm$ 3%. Thus, the mean change in Δ/mol per unit of U/S (i.e. the mean of the α-tocopherol induced changes in Δ/mol for all ages divided by the mean of analogous changes in U/S) was smaller in PC than in PE; specifically, a value of 0.031 was obtained for PC while a value of 0.058 was obtained for PE. Since the melting point of lipids is largely dependent on Δ/mol, these results suggest that the "fluidity" of PC changed less than that of PE as a consequence of α-tocopherol treatment. PC presently is believed to be located in the outer half of the bilayer of biological membranes while PE is thought to be located primarily on the inner half of the bilayer. Among samples of PC of the five ages, both from treated and control cultures, 18:3 decreased between 48 and 96 hours of age but increased between 96 and 120 hours. In PE, however, 18:3 decreased continually between 48 and 120 hours. These data indicate that α-tocopherol may slow but may not prevent removal of 18:3 from PC and PE during aging of *G. cingulata*.

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RELATIONSHIP BETWEEN LIPOPEROXIDE AND AGING. Kazuo Fukuzumi, 11 Nunoike-cho, Higashi-ku, Nagoya, Japan.

The lipoperoxide theory is proposed to explain aging through the free-radical and cross-linking theories combined. Lipoperoxide theory states aging is generated by lipoperoxides in a living body. The author described the phenomena of aging in the papers on lipoperoxides and geriatric diseases published in 1965 and 1969. By the reaction with lipoperoxides, the –SH group of Coenzyme A necessary for β-oxidation changes into inactive –S–S– group. Under the presence of lipoperoxides, β-oxidation becomes inactive, and the autoxidation of lipids generating lipoperoxides, (the competing reaction with β-oxidation) proceeds easily. Thus, lipoperoxides are accumulated in a living body. Under "lipoperoxide theory", aging may advance. Almost all phenomena about aging,– wrinkles, senile pigment, presbyopia, decreases of moisture outside cells, total moisture of a living body, mucopolysaccharide, cell number, and a lowering of organ function–can be elucidated by "lipoperoxide theory:" (1) Peroxy radicals are made from lipo-

peroxides, and these active radicals produce protein radicals, such as collagen. (2) In a comparison of protein and the substance containing the –OH group, such as water, hydrogen bonds between protein radicals and the substance become weaker, and the cross-linking between these protein radicals is facilitated. (3) By steric hindrance, the hydrogen bond between this cross-linking product and the substance containing the –OH group, such as water, is prevented. Aging phenomena described before can be explained reasonably, by bringing steps 1, 2 and 3 into focus.

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LIPOFUSCIN PIGMENT AND LYSOSOMAL ENZYMES IN AGING RAT LIVER CELLS. D.L. Knook, Ph.D., C.F.A. van Bezooijen, Ph.D. and E.Ch. Sleyster, Institute for Experimental Gerontology TNO, Lange Kleiweg 151, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands.

The progressive accumulation of age pigment (lipofuscin) in non-replaceable postmitotic cells of many organs (including brain, heart and liver) is an age-correlated process. The lipofuscin granules are probably residual bodies derived from lysosomes and they have been found to contain several lysosomal hydrolases. The chemical structure of lipofuscin is heterogeneous, but fundamentally lipid in structure. It consists of a mixture of polymeric lipid and phospholipid structures, along with amino acids. The initiating reaction in the formation of lipofuscin is believed to be polyunsaturated lipid peroxidation. A study of the functional consequences of lipofuscin accumulation is still an unexplored area of research. For this type of study, a useful experimental model system is isolated hepatocytes prepared from rat liver. Under normal in vivo conditions, these cells do not divide, live as long as the animal and accumulate lipofuscin during the lifespan of the rat. After isolation the cells retain their functions. Variations in the amount of lipofuscin in vivo naturally as a consequence of aging, or experimentally by the use of several drugs, make possible a study of the relationship between lipofuscin content and lysosomal and cellular functioning. With age, an increased lipofuscin content in hepatocytes from 32 to 34-month-old rats was found to be accompanied by a diversity of changes in lysosomal enzyme patterns. The specific activity of proteolytic enzyme cathepsin D was increased greatly, whereas that of aminopeptidase B decreased. Treatment with the drug centrophenoxin reduced the amount of lipofuscin in the hepatocytes and caused short-term, reversible changes in the activities of several lysosomal enzymes. The effect of lipofuscin induction or reduction on hepatocyte functions such as bromsulphthalein storage and albumin and protein synthesis was studied with isolated cells. These functions showed clear age-dependent alterations.

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MESENCHYMAL HYPOTHESIS OF THE PATHOGENESIS OF ARTERIOSCLEROSIS. W.H. Hauss, Universität Münster und Ehrenvorsitzender des Instituts für Arteriosklerosеforschung an der Universität Münster, BRD, Germany.

Three main fields of events or factors are suspected of being involved in the pathogenesis of arteriosclerosis and atherosclerosis: disturbances of lipid metabolism, of blood coagulation and of arterial wall cells. Mesenchymal cells—previously considered to be bradytrophic—have on the contrary a very active metabolism and moreover react sensitively, regularly, and promptly to many irritating and injurious factors of various kinds by a change in their metabolism, to give a rise to what we have called the "non-specific mesenchymal reaction." Experiments in animal models and with cultivated arterial wall cells give evidence, that the reaction of the arterial wall cells on pathogenic factors is an essential event in the pathogenesis of arteriosclerosis. Generally accepted risk factors such as arterial hypertension, diabetes mellitus, atherogenic diet and toxins, are demonstrated to induce changes of the cell proliferation rate, of the synthesis of proteoglycans, of collagen, of lipid acids, and of cholesterol. This reaction of the arterial wall cells induces the primary pathologic histological structures in the arterial wall: intima edema, hyalinosis, and fibrosis, and participates considerably in producing the lipidosis, necroses, and thrombosis. Just as in rheumatic arthritis, the pathologic metabolism of synovial cells, which are mesenchymal cells, deforms the connective tissue of the joints. The pathologic metabolism in the arterial wall cells, which are also mesenchymal cells, produces the histologic deformations of the arterial wall structure. The knowledge of the similarity of the nature of the rheumatic and the arteriosclerotic diseases not only emphazises the essence of sclerogenesis, but also suggests how to enlarge our arteriosclerosis therapy.

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PRODUCTION AND USE OF NATURAL ANTIOXIDANTS. U. Bracco, J. Löliger and J.L. Viret, Nestle Products, Technical Assistance Co. Ltd., P.O. Box 88, CH-1814 La Tour-de-peilz, Switzerland.

We developed a new industrial process to obtain natural antioxidants from spices and other vegetables, without using any solvent extraction, by adapting mechanical and physical treatments. It is known that several natural spices show antioxidant properties which could be applied to extend the shelf life of food. Rosemary, sage, paprika and nutmeg were submitted to a mechanical treatment (micronisation) and the finely powdered spices extracted further with an edible vegetable oil, i.e. groundnut. The antioxidant dissolved in the lipid phase is collected by double-steps fall film molecular distillation allowing separation of the lipid phase (to be recycled) from the active low molecular weight fraction. The antioxidant activity has been measured on fats, oils and several fat-containing foods by the oxygen absorption method. In this latter case, head space analysis (i.e. pentane) and organoleptic evaluations showed the antioxidant activity of molecular distillates, and indicated rosemary derivatives protect foods against oxidative rancidity.

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NATURAL ANTIOXIDANTS ISOLATED FROM CLOVE. Roslyn E. Kramer, Food Sciences Laboratory, US Army Natick R&D Command, Natick, MA 01760.

Ground clove was extracted with petroleum ether and 80% ethanol by percolation technique. The ethanol extract was further fractionated with ethyl acetate and then ethyl ether. Antioxidant activity was greatest in the ethyl acetate fraction. This was analyzed using thin layer chromatography (TLC) on polyamide flexible sheets and developed with chloroform/methanol 1:1. The spots were inspected under ultra violet (UV) light and visualized by spraying with α,α-dipyridyl and ferric chloride. A test to identify antioxidants directly on the TLC sheet was used and on subsequent sheets that area identified as antioxidant was scraped off and extracted. Gallic acid was tentatively identified by UV, infrared (IR) and mass spectrometry (MS) as a major component of this fraction. The R_F value of this compound on TLC was the same as that of gallic acid and visualization results were identical. The sample was purified using high pressure liquid chromatography and again run on IR and MS for verification.

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POLY AO^{TM}-79—A NEW ANTIOXIDANT FOR USE IN FOOD. Ned M. Weinshenker, Steven Goldby and Thomas M. Parkinson, Dynapol, 1454 Page Mill Road, Palo Alto, CA 93125.

The functional utility and safety testing of a new, nonabsorbable antioxidant for food use are described. Poly AO^{TM}-79 has been in development at Dynapol for five years. It has been tested under a modern and comprehensive safety testing program and has been proven to be safe. The polymeric nature of this product provides the safety advantage of being nonabsorbable and the functional advantage of being nonvolatile and only slowly diffusing out of food systems. These properties significantly increase the carry-through in a variety of processing conditions. An overview of the results of safety tests, along with highlights of functionality data, will be presented. Submission of a food additive petition to the FDA is scheduled for the spring of 1980.

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ON A CATALYTICAL INFLUENCE OF THE SELECTED AMINO-ANTIOXIDANTS AT THE PROPAGATION STATE OF AUTOXIDATION OF OLEYL ALCOHOL. Teresa Kowalska, Ph.D., Institute of Chemistry, Silesian University, 9, Szkolna Street, 40-006 Katowice, Poland.

In studies on the influence of interactions through H-bonds at the propagation stage of autoxidation of oleyl alcohol, the results of continued experiments deal with the influence of the selected amines at the propagation stage of autoxidation of the same compound. It was established that at the early period of propagation, the amino-antioxidants showed the catalytical properties due to their ability to interact through H-bonds with the peroxidic RO_2 radicals, derived from oleyl alcohol. Only after certain period of time, i.e. after having accumulated a sufficient amount of the In$^{\cdot}$ and H$^{\cdot}$ radicals in a given sample according to the reaction course: InH→In$^{\cdot}$ + H$^{\cdot}$, the applied inhibitors started demonstrating their proper role—retarding autoxidation. On this basis a conclusion was drawn that the inhibitors, including active hydrogen atom(s), in a molecule act both as catalysts (in a molecular form) and antioxidants (in a radical form) of autoxidation. The kinetical values obtained with their help reflect certain compromise between these two functions.

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ANTIOXIDANTS AS MULTI-FUNCTIONAL PRESERVATIVES IN FOOD AND COSMETICS. Jon J. Kabara, Department of Biomechanics, A420 East Fee Hall, Michigan State University, East Lansing, MI 48824.

In recent years more and vigorous governmental regulations have increased responsibility in the pharmaceutical, cosmetic and food industries. In no other area is this more true than in the use of chemical preservatives. No longer should we continue to add chemicals to attain specific characteristics in a product. We must look for those chemicals which when added to a product will have multiple

functions. Many well known antioxidants fulfill this requirement. Tert-butyl-hydroxy-anisole (BHA), tert-butyl-hydroxy-toluene (BHT) and nordihydroguaiaretic acid (NDGA) are food additives which not only function as antioxidants but have been shown to be reasonably good antimicrobial agents. These additives have wide spectrum activity. A number of BHT derivatives have been studied and their structure related to antimicrobial activity. Of the n-alkyl derivatives tested the butyl chain length was found to be most active against bacteria and even viruses. Since most antioxidants are lipid soluble, their emulsification in products is also important. The use of lauricidin, a GRAS emulsifier, not only aids the dispersion of the phenolic biocides but also is additive or synergistic to its antimicrobial properties. Thus, antioxidants and specific lipids can be combined to achieve high chemical and biocidal preservative action. The application of such preservative systems as outlined above obviates the use of many chemicals formerly added individually to achieve the same objective. Our proposal will not add more antioxidants to the consumer product but will make better use of the multi-functional properties of various chemicals. By the judicious choice of preservative systems we will achieve greater consumer benefits at lower risks.

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CATALYTIC ACTION OF SOME INORGANIC FOOD ADDITIVES ON THE OXIDATION OF 2, 6-DI-TERT-BUTYL-4-METHYLPHENOL (BHT). Beatrice Leventhal, Henryk Daun and Seymour G. Gilbert, Department of Food Science, Rutgers—The State University, Box 231-Cook College, New Brunswick, NJ 08903.

Aluminum oxide, silicic acid and carbon black are used in foods as carriers of active ingredients, colors, dispersants, anti-caking agents, in packaging materials, and for other purposes. Our experiments show that these compounds act also as catalysts in the oxidation of 2, 6-di-tert-butyl-4-methylphenol (BHT), a widely used antioxidant. BHT was mixed in various proportions with aluminum oxide, silicic acid and carbon black (1:1, 1:10, 1:100) and held at 50C and 75C for 14, 28 and 65 days. Each sample was extracted with acetone. The extracts, subjected to gas chromatographic analysis, showed several peaks with the same retention time but significantly different peak areas in various samples. Thin-layer chromatography indicated several R_f values similar for all samples but with quantitative differences dependent upon the experimental conditions. R_f of several yellow spots correlated with values for BHT oxidation products reported in other publications. One of the yellow spots was identified as 3,3′,5,5′-tetra-bis-tert-butyl-stilbenequinone. Data indicated that aluminum oxide, silicic acid and carbon black catalyze the oxidation of BHT below and above its melting point (70C). The results of our research provide an explanation for discolorations observed in a large variety of products containing BHT and aluminum oxide, or silicic acid or carbon black.

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BASE SYNERGISM OF THE ANTIOXIDANT ACTIVITY OF CATECHOLAMINES. P.A.T. Swoboda, G.R. Fenwick and L.J. Parr, A.R.C. Food Research Institute, Colney Lane, Norwich, NR4 7UA, England.

Cocoa solids have long been considered to possess antioxidant activity. Recently salsolinol (1-methyl-6, 7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) has been identified as a constituent. This catecholamine would appear from its structure to be a potential antioxidant of natural origin and therefore we have studied its activity in a model system. Antioxidant activity was measured in dry lard at 100C using a manometric system to detect the end of the induction period and the onset of rapid oxygen uptake. Although salsolinol salts were found to be inactive, the free base proved to be a powerful antioxidant when tested at the 200 ppm level. The activity of a series of compounds with related chemical structures was investigated also. Mixtures of salsolinol hydrochloride with mono-sodium phosphate, di-sodium phosphate, or tri-sodium phosphate (phosphates which are permitted E.E.C. additives) showed a progressive increase in induction period; the mixture containing mono-sodium phosphate was inactive, while that containing tri-sodium phosphate approached a hundredfold protection factor.

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APPLICATIONS OF ANTIOXIDATIVE MAILLARD REACTION PRODUCTS IN FOODS. Hans Lingnert, SIK—The Swedish Food Institute, Fack, S-400 23 Göteborg, Sweden.

Maillard reaction products from reducing sugars and certain amino acids or protein hydrolysates have been shown to have antioxidative effect in model systems. If the antioxidative properties of Maillard reaction products are to be used in foods, two essentially different methods of application are possible: (1) potent antioxidants, synthesized from the appropriate sugars and amino compounds and possibly purified by fractionation, can be used as a normal food additive; (2) since the Maillard reaction is common in food processing, the formation of *antioxidative* Maillard reaction products may be optimized in many foods by controlling the process parameters and the recipe. The appropriate protein hydrolysates, amino acids or sugars can be included in food recipes, which are normally subject to sufficient heat treatment. Both methods of application were tried in storage experiments with foods. The storage stability of cookies was improved by adding histidine and glucose to the dough. Maillard reaction products formed during the baking were probably responsible for the antioxidative effect. During frozen storage of sausage, preformed Maillard reaction products added to the sausage batter were shown to retard lipid oxidation. Maillard reaction products from histidine and glucose as well as Maillard reaction products from enzymic hemoglobin hydrolysate and glucose were found to be effective.

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BEHAVIOUR OF ETHOXYQUIN ANTIOXIDANT IN FISH MEAL. A.A. Spark, Fishing Industry Research Institute, University of Cape Town, Private Bag, Rondebosch 7700, South Africa.

Ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2 dihydroquinoline) (EQ), is used as an antioxidant in many fish meals to preserve energy value and to help prevent self-heating. The EQ disappears during the first months of storage, but the fish meal remains stable. The determination of EQ, and proof that any fish meal sample was treated, is of great concentration to the fish meal industry. Work in this sphere has recently been restarted and some interesting observations made concerning the manner in which EQ disappears in fish meal. In several samples, cyclic behavior was observed with evidence that EQ is converted to a more potent antioxidant, and vice versa. This other antioxidant may be EQ nitroxide. There is also evidence that EQ triggers the formation of natural unidentified antioxidants in the fish meal, and a possible explanation for apparently stable meal heating up in storage is presented. This is releated to a threshold minimum of EQ, which appears capable of triggering oxidation.

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THERMAL BEHAVIOR OF ORDERED AND DISORDERED CRYSTALS FROM FATTY ACIDS, ESTERS, AND ALKANES. Shu-Pei Chang, Northern Regional Research Center, AR/SEA, USDA, 1815 N. University Street, Peoria, IL 61604.

Mono- and dienoic fatty acids, C_{18-12}, and their methyl esters exhibit multiple endotherms and exotherms when frozen and melted in a Perkin-Elmer DSC-2. Corresponding saturated acids, esters, and alkanes generally produce single, sharp endotherms and exotherms that differ by only a few degrees for each material. Related trienoic and tetraenoic compounds, in which unsaturation disrupts zig-zag structure, also exhibit single though broad peaks; of the materials examined, these compounds have the lowest temperatures and heats of transition. When cooled rapidly to prevent ordered crystallization, the unsaturated compounds exhibit freezing exotherms; but when reheated, the compounds also undergo additional exothermic disorder/order transformations of the sort common to certain high polymers and some simple organic compounds. These transformations reflect the sample's thermal history; rapid quenching increases the number and sharpness of the exothermic peaks and lowers their temperatures; tempering removes one or all of the exotherms without affecting the melting endotherms. These exothermic orientations account for energy differences between melting endotherms and freezing exotherms for the same sample and generally represent about 30% of the heat of fusion. It thus constitutes a significant property. As yet, calorimetric data allow no clear choices between models that could account for this behavior, but the property offers appealing prospects for kinetic analyses of crystallization.

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THE ELECTRON MICROSCOPE LOOKS AT LIPIDS. Susan Jones, USDA, AR, SEA, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Aspects of the supramolecular organization of phospholipids have been imaged by electron microscopy. Freeze-fracturing cleaves a frozen specimen so that a replica of the fractured surface can be observed through the microscope. This technique has provided unique information about pure, hydrated phospholipids and lipid-protein-water systems and has contributed greatly to advancement in the understanding of biological lipid-protein membranes. Visualization of extended regions of the exterior and interior of lipid bilayers permits detailed study of the spatial arrangement of intramembrane protein particles. Evidence from freeze-fracture indicates that lateral phase separation of lipids in cell membranes is accompanied by aggregation of membrane proteins. Some membranes show close correlation between onset temperatures for lipid phase transition and the temperatures that lead to particle aggregation. In mixtures of certain pure, hydrated phospholipids undergoing phase transition, freeze-fracture clearly shows the presence of two-dimensional solid domains as well as fluid regions. This technique is powerful when used to complement physico-chemical methods such as DSC, X-ray diffraction, ESR, NMR, and fluorescence spec-

troscopy that give data reflecting averaged conditions in the bilayer. Other preparative methods for viewing phospholipid bilayers by electron microscopy are thin sectioning and negative staining. These are based on the property of hydrated phospholipids to accumulate heavy metal stains at the hydrophilic surfaces.

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ELECTRON MICROSCOPY OF SOYBEAN LIPID BODIES. Craig W. Bair and Harry E. Snyder, Frito-Lay Div., Pepsi Co. Inc., 900 N. Loop 12, Irving, Texas 75061.

Soybean oil is an economically important product, but knowledge of the oil in situ and of conditions affecting the oil is surprisingly limited. We made an electron microscopic study of the lipid bodies (a term preferred over spherosomes) in situ and after isolation; we also analyzed lipid bodies isolated by different procedures. The results showed that soybean lipid bodies are small (approximately 0.2 to 0.5 μm in diameter) with an affinity for cell membranes, protein bodies, endoplasmic reticulum, and other cell organelles but not for mitochondria or nuclei. Isolation of lipid bodies in 0.5M saline with extensive washing gave the highest lipid content and lowest protein, but still there was 15% protein in the isolated lipid bodies. They had membranes about half the thickness of a unit membrane, and treatment with trypsin caused breakdown of the membranes. The isolated lipid bodies had a range of densities from less than 1.0066 to 1.0788 determined by sucrose density gradient centrifugation. Based on the range of densities and on the distribution of lipid bodies in floating, supernatant and sedimenting layers, we concluded lipid bodies are surrounded by a specific delimiting membrane but can have a variable amount of randomly adhering protein associated.

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CRYSTALLIZATION PROPERTIES OF HYDROGENATED CANOLA OIL. D.K. Loewen, Director of Research and Quality Control, CSP Foods Ltd., P.O. Box 190, Saskatoon, Saskatchewan S7K 3K7 Canada.

Recent studies on the crystallization phenomenon of canola oil and the quality of finished products will be described and discussed in relation to the nature and history of the oil, the composition of finished products and solutions to the problem. A detailed 24-week study using polarized light photo micrographs will trace the crystal development, from the alpha to the beta stages, using canola oil from the variety tower. Recent studies and developments on the varieties candle, regent and altex will be discussed as well as an attempt to explain the beneficial crystal stabilization effect of sorbitan tristearate.

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EFFECT OF PHYSICAL PROPERTIES OF PLASTIC FATS ON THERMAL STABILITY AND MECHANICAL PROPERTIES OF FAT-PROTEIN GEL PRODUCTS. C.M. Lee and J.W. Hampson, Dept. Nutrition & Food Sciences, Drexel University, Philadelphia, PA 19104.

Thermal destabilization of meat emulsions has been frequently reported. In this study we show how the physical properties of plastic fats influence the thermal stability and mechanical properties of such emulsions after cooking. Meat was comminuted with soybean oil-based plastic fats of different physical properties and cooked at two different rates. Thermal stability of the emulsion, as measured by fat and water retention, was inversely related to fat softness, melting properties, and heating rate. Good stability was achieved when the solids content (determined by DSC) in the total fat was increased to 50% (22% product fat content) and to 60% (27% product fat content–uncooked weight basis). Release of fat and water upon heating commenced about 10C below the softening point of the fat. The physical state of the protein gel matrix was evaluated by the amount of expressible fluid obtained under compression and by the change in mechanical properties under uniaxial compression and shear. Compressive strength (CS) increased markedly and shear strength (SS) increased moderately with increasing hardness of fat. Both CS and SS reached a maximum at the 50% fat solids and 60% fat solids levels. Mechanical strength of cooked product was inversely related to the amount of expressible fluid. Increased fat levels tended to reduce mechanical strength. Faster heating resulted in greater mechanical strength of cooked product but caused fat separation in high fat products formulated with soft fats.

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EFFECT OF FREE FAT ON RECONSTITUTABILITY OF DEHYDRATED DAIRY PRODUCTS. V.H. Holsinger, USDA, ERRC, 600 East Mermaid Lane, Philadelphia, PA 19118.

Many physical properties pertaining to rehydration of dehydrated lipid-containing milks and milk analogues are dependent on the physical state and distribution of the lipid, especially the free fat (unemulsified fat) content. Free fat has been associated with poor dispersing properties and scum formation upon reconstitution of dry whole milks. A major influence on free fat content is the drying method selected because of its effect on physical structure of the powder particle, dominant features being spatial distribution of the fat globules on the surface and within the powder particle, particle size distribution and particle porosity. Conventional spray drying results in lowest levels of free fat because of low particle porosity and uniform distribution of fat droplets throughout the particle. Small particle fractions contain more free fat than the main body of the particles due to more fat on the surfaces. Increased free fat content decreases powder wettability and dispersibility on reconstitution. Reconstitutability is improved by rehydrating the powder at temperatures above the melting point of the lipid. Hydrophilic surfactants added before the drying step enhance wettability and dispersibility but can cause fat churning during reconstitution. Powder wettability has also been related to the physical state of the lipid. Wettability is decreased in powders stored at room temperature, coinciding with milkfat crystallization; rapid cooling after drying and cold storage improve wettability. If the lipid phase is liquid, the powder is more wettable; powders dried with low melting fractions of milkfat or with liquid vegetable fat have improved wettability and dispersibility. Scum formation was also reduced by liquefying the lipid phase.

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POSITIONAL DISTRIBUTION OF THE FATTY ACIDS WITHIN THE TRIGLYCERIDES OF MANGO (*MANGIFERA INDICA*) KERNAL FAT. W. Van Pee, L. Boni, M. Foma, M. Hoylaert and A. Hendrikx, Lab. of Tropical Food Production & Technology, Catholic University of Leuven, Kardinaal Mercierlaan 92, B-3030, Heverlee, Belgium.

Recent work on the processing of the stones of mango fruit (*Mangifera indica*) has shown that the kernels of mango fruit represent a potential source of fat. We analyzed the triglycerides of the seed fat from nine different mango varieties. The main fatty acids of the triglycerides are stearic and oleic acid, which respectively represent, according to the variety, 32.4 to 44.0% and 43.7 to 54.5% of the fatty acids. The remainder fatty acids are palmitic acid, 5.9-9.1%, linoleic acid, 3.6-6.7%, linolenic acid, less than 1.6%, and arachidic acid, less than 0.7%. Analyses of the positional distribution of the fatty acids within the tryglycerides of the fat showed that palmitic, stearic and arachidic acid are almost exclusively incorporated at the sn-1- and sn-3-position. The oleic and linoleic acid content at the sn-2-position are similar for the different varieties, i.e. 87.3% for oleic acid and 10.4% for linoleic acid. The amount of the saturated fatty acids, i.e. palmitic and stearic acid, and of oleic acid, incorporated at the sn-1-and sn-3-position are linearly related to their respective content in the total triglycerides.

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UTILIZATION OF SAL FAT AND MOWRAH FAT FOR THE PREPARATION OF PLASTIC FAT PRODUCTS BY INTERESTERIFICATION REACTIONS. D.K. Bhattacharyya, R.S. Vaidyanathan, M.M. Chakrabarty and K. Kar, Department of Applied Chemistry, University Colleges of Science and Technology, 92, Acharyya Prafulla Chandra Road, Calcutta 700009, India.

Sal (*Shorea robusta*) and mowrah (*Madhuca latifolia*) are the two major oil seed crops of tree origin in India. The fat from these seeds has been recognized as suitable raw material for the preparation of confectionery, bakery and other kinds of plastic fats after suitable modifications. The present study is aimed at the use of sal and mowrah for the purpose of preparing plastic fats for edible uses by ester-ester interchange and acidolysis reactions. The ester-ester interchange reaction involving randomization of sal and mowrah blended with a suitable fatty material and the acidolysis reaction of sal and mowrah with selective fatty acids yield products of various chemical compositions and physical properties. Some of the products can be used directly as food fats and some after fractionation from a solvent. The conditions of the reactions and the results will be discussed.

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FATTY ACID VARIABILITY OF PLASMA CHOLESTERYL ESTERS IN ADULT TWINS. Vijay Warty, Frank Harmath, Ke Won Kang, James A. Norton, Jr. and Joe C. Christian,* Medical Genetics, I.V. Medical Center, 1100 W. Michigan Street, Indianapolis, IN 46223.

Genetic variation of the fatty acid fractions of plasma cholesteryl esters was studied using 79 sets of twins (42 monozygotic (MZ) and 37 dizygotic (DZ) pairs) who ranged in age from 18 to 31 years. Thin layer chromatography was used to separate cholesteryl esters from other plasma lipids. The fatty acids from lipid extracts of cholesteryl esters were converted to methyl esters and then separated by gas chromatography. The predominant fatty acids obtained were palmitic (16:0), oleic (18:1) and linoleic (18:2). In general, MZ twins were characterized by smaller within mean squares than DZ twins. Only linoleic acid (18:2) had a significant estimate of genetic variance within pairs. This work was supported by Grant No.

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ORGAN PIPE CACTUS STEROL DIOLS AS HYPOCHOLESTEROLEMIC AGENTS. Henry W. Kircher and Cynthia McNulty, Dept. of Nutrition and Food Science, University of Arizona, Tucson, Arizona 85721, and Andrew A. Kandutsch, Jackson Laboratory, Bar Harbor, Maine.

Some oxygenated sterols are inhibitors of cholesterol biosynthesis; they may repress synthesis of HMG-CoA reductase. Outer tissues of organ pipe cactus are rich in lipids (12-15% of dry weight) that contain about 20% 3β, 6α-sterol diols in both the free and esterified state. To determine if the diols had evolved as deterrents to rodent predation, they were administered to young rats by injection and in a cholesterol-free diet. In contrast to 15-oxygenated sterols, the organ pipe sterol diols had no effects on food consumption, weight gain or plasma cholesterol when they were fed as 0.1% of the diet. Rats injected subcutaneously in the neck with 5 mg of the diols in 0.25 ml olive oil daily for 6 days ate less and gained less weight than those injected with 5 mg cholesterol in the oil or olive oil alone, but again, no differences were noted in the levels of plasma cholesterol between the three groups. Nodules formed at the site of injection in the diol treated rats and this correlated with a high immunoglobulin titer (42 mg/ml) in this group when compared to the other two (14-16 mg/ml) or untreated rats (13 mg/ml). The activity of the organ pipe sterol diols as suppressors of cholesterol synthesis and HMG-CoA reductase in L-cell (mouse fibroblast) cultures was in the range exhibited by previously tested 6-oxygenated sterols. The evidence suggests that the elaboration of a 6-hydroxylation pathway in organ pipe cactus sterols was not a response to predation by rodents.

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EFFECTS OF PROBUCOL ON CHOLESTEROL METABOLISM IN THE RAT. Job R. Li, Northwest Lipid Research Clinic, 326 Ninth Avenue, Seattle, WA 98104, and Rosemary J. Holets and Bruce A. Kottke, Mayo Clinic/Foundation.

Probucol (PB) effectively reduces plasma cholesterol levels in experimental animals and humans. The mechanism of the hypocholesterolemic action of PB has not yet established. The present study further explores aspects of the nature of the cholesterol-lowering effect of PB in rats. Male Sprague-Dawley rats were divided into three groups: one group was given 0.25% PB containing laboratory chow diet (LC), one group was given 0.25% (w/w) clofibrate (CF) containing LC diet and the third group was fed LC diet only. All groups of rats were maintained on these regimens for 4 weeks before sacrifice. Plasma cholesterol levels, hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities and fecal excretion of sterols were determined in these animals. At the end of 4 weeks, PB-treated rats had lower plasma cholesterol levels than the LC (36.2±2.9 vs 51.3±2.9, $0.01 < p < 0.02$). Similarly, CF-treated rats had a reduced plasma cholesterol level. PB treatment significantly reduced cholesterol 7α-hydroxylase activity (13.9±1.6 vs 22.2±2.4, pm/min/mg, $0.02 < p < 0.010$) and CF treatment resulted in a reduction of HMG-CoA reductase activity (16.8±3.0 vs 29.3±2.6 pm/min/mg, $0.001 < p < 0.005$) and cholesterol 7α-hydroxylase activity (14.7±1.9 vs 22.2±2.4, pm/min/mg, $0.02 < p < 0.025$). The fecal excretion of both neutral and acidic sterols were significantly reduced in the PB or CF treated rats. The present studies indicate that probucol treatment in rats caused a reduction in plasma cholesterol level, fecal excretion of sterols and suggests that mechanism for hypocholesterolemic action of probucol appears to differ from that of clofibrate.

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THE EFFECT OF DIFFERENT DIETARY FATS ON CHICK PERFORMANCE AND ENERGY UTILIZATION. Remi De Schrijver, The Hormel Institute, University of Minnesota, 801 - 16th Avenue N.E., Austin, MN 55912.

In feeding experiments carried out with broiler chicks between the ages of 0-6 weeks, fat-unsupplemented control diets were compared with diets containing 0.5% and 10.5% added soybean oil, lard and tallow. Significant linear relationships were calculated between the level of supplemented fat and the improvement of feed conversion and growth as compared with the control animals. The most favorable results were obtained with the soybean oil diets, followed by the lard and the tallow diets. In balance experiments a control diet and diets containing 4.5% and 8.5% of supplemented soybean oil, lard and tallow were used. Chicks were kept from 2 to 4 weeks of age. Use of gross energy intake for growth was higher as the dietary fat level increased. Chicks fed soybean oil diets showed highest use of gross energy intake for growth followed respectively by chicks consuming lard and tallow. It was found that the metabolizable energy was more efficiently used for tissue energy gain as the dietary fat content increased. It could be concluded that during the metabolic processes the energy lost as heat from non-fat compounds is higher than the heat loss from the added fats. The supplemented dietary fats did not exert a significantly different effect on the net availability of the metabolizable energy for growth, although a slightly higher efficiency was observed with the soybean oil diets. Analog results were also obtained in chick respiration experiments in which diets containing 4.5% lard and 4.5% soybean oil were compared. Contradictory to some past results, the experiments showed that the metabolizable energy of soybean oil is higher than the metabolizable energy of two animal fats and that lard has a higher metabolizable energy value than tallow.

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LIPOPROTEIN PATTERNS IN TWO STRAINS OF MALE ICR MICE DEVELOPED BY SELECTION FOR HIGH AND LOW TOTAL SERUM CHOLESTEROL CONCENTRATIONS. L.F. Ferreri, V.P.I. & S.U., Blacksburg, VA, E.A. Dunnington, West Virginia Univ., Morgantown WV, and J.M. White, V.P.I. & S.U., Blacksburg, VA.

ICR albino mice were bred through 14 generations. Selection for total serum cholesterol concentrations produced a high cholesterol strain (CH), 431.84 ± 12.32 mg/dl, and a low cholesterol strain (CL), 57.08 ± 1.86 mg/dl. Lipoprotein profiles of male mice were examined by gel filtration (Biorad A5M) on pooled sera and by agarose electrophoresis on individual sera. Gel filtration profiles of pooled sera showed all three peaks (I-VLDL + chylomicrons, II-LDL, III-HDL) were higher in CH mice than in CL mice by 66.7%, 55.0% and 32.8% for peaks I, II and III, respectively. Lipoprotein content was estimated by calculation of areas under the absorbance peaks (280 nm). A difference in lipoportein distribution was also evident. CL serum contained 15.5% I, 11.0% II and 73.5% III, whereas CH serum contained 25.7% I, 13.8% II, and 60.5% III. Thus, total lipoprotein and all three lipoprotein fractions increased in CH mice; however, the percentage of HDL relative to other lipoproteins was less in CH mice. Electrophoretic analysis revealed further alterations in HDL. HDL of CL mice consisted of a double band; a diffuse α-migrating band adjacent to a slightly faster "sharp" α band. In CH mice a marked increase in stainable material was present in the diffuse α-band. The results demonstrate distinctive alterations in quantitative and qualitative lipoprotein patterns which reflect changes in lipoprotein metabolism as a consequence of genetic selection.

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POLYUNSATURATED OILS AND PYRUVATE DEHYDROGENASE ACTIVATION. Claude Loriette, Micheline Launay and Jeanine Raulin,* Laboratoire Nutrition Cellulaire & Lipophysiologie, Université Paris 7 and C.N.R.S., 2 Place Jussieu, 75221 Paris Cedex 05, France.

The rate of 1-^{14}C pyruvate decarboxylation was measured on crude brain and liver homogenates using an assay system adapted from Wieland et al. (1971) with 10 mM $MgCl_2$ preincubation for full activation of the enzyme. Female rats were kept on a fat-free diet (FF) from the 10th day after mating until weaning. Pyruvate dehydrogenase complex (PDHc) total activity was found to be very low in brains and livers of their progeny when the weaned rats were maintained on the FF diet. PDHc activity increased dramatically in the LP group of brains and livers due to the addition of a limiting amount (0.7%) of sunflower oil. Traces of C20:3 were still visible 7-14 days after weaning suggesting that the animals did not recover entirely from the maternal lipid deprivation although all the LP rats seemed to be healthy. Conversely, 20% sunflower oil (SO diet) did not improve PDHc activity. Ten days after weaning, no difference in PDHc activity was found in brains of the SO group compared to the FF group, while PDHc activity was significantly lower in SO livers than in FF livers. The presence of C20:3 in brain and liver lipids was not detectable after 7 days on an SO diet. In maternal livers, PDHc activity was also increased by the small amount (0.7%) of sunflower oil supplied in the LP diet when this was given for one month after the end of suckling. The LP diet was however ineffective in increasing PDHc activity in maternal brains while the SO diet containing 20% sunflower oil significantly increased PDHc activity.

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INCORPORATION OF AN ESSENTIAL FATTY ACID (LINOLEIC) OR A *TRANS* FATTY ACID (ELAIDIC) AND THEIR METABOLITES INTO PHOSPHOLIPIDS OF CULTURED HUMAN FIBROBLASTS. R.E. Pitas, T.L. Innerarity and R.W. Mahley, Gladstone Foundation Laboratories, P.O. Box 40608, San Francisco, California 94140.

Human fibroblasts were grown for 5 days with culture medium containing 4% lipoprotein-deficient serum (control) or similar media supplemented with 20 μg per ml of elaidic acid (18:1 *trans*) or linoleic acid (18:2ω6), as the fatty acid-albumin complex. The cellular phospholipids were isolated and their fatty acid compositions determined by capillary column gas-liquid chromatography. Supplementation of the media with 18:1 *trans* resulted in its incorporation into all of the phospholipid classes (cells grown in control media contained only trace amounts of this fatty acid). In phosphatidyl-

choline (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) the 18:1 *trans* content was increased to 55-60% of the total fatty acids with a reduction in the 16:0, 18:0, and 18:1 *cis*. In sphingomyelin (Sph), 18:1 *trans* (28%), as well as its chain elongation 24:1 *trans* (10%), and oxidation 16:1 *trans* (6.7%) products, were incorporated at the expense of 16:0 and 24:1 *cis*. In cells supplemented with linoleic acid, 18:2ω6 was incorporated into PC, PE, PS and PI in amounts varying from 15-30% (control value 1 to 2%) with a decrease in 18:1 *cis* and an increase in 20:3ω6 (5 to 15 times control values). It was of interest that the percentage of 20:4ω6 (the desaturation product of 20:3ω6) was either not changed or reduced. In Sph, 18:2ω6 (supplementation resulted in a decrease in 16:0 and 24:1 *cis* and an increase in 24:2ω6 (38% vs. 3% of the control). Isolation of ^{14}C-24:2ω6 following supplementation with ^{14}C-18:2 demonstrated that 24:2ω6 was formed by chain elongation of 18:2ω6. The data show that cultured normal human fibroblasts incorporate and metabolize 18:1 *trans* and 18:2ω6. The cells desaturated (Δ6 desaturase) and elongated 18:2ω6 to 20:3ω6. In addition, the cells elongated both 18:1 *trans* and 18:2ω6 to 24:1 *trans* and 24:2ω6, respectively, and incorporated these products into sphingomyelin. The fatty acid composition of all the phospholipid classes was substantially altered by addition of specific fatty acids to the culture media.

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α-LINOLENIC AND LINOLEIC ACIDS AND THE IMMUNE RESPONSE IN THE LEWIS RAT. L.A. Marshall and P.V. Johnston, 205 Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, IL 61801.

The functional significance of α-linolenic acid and its metabolites is unclear. The docosahexaenoic metabolite, 22:6ω3, is concentrated in certain tissues such as the brain, muscle, heart, and testes. In the lymphocyte 22:6ω3 is concentrated in the phosphatidylethanolamine fraction. The proliferative and functional capacity of lymphocytes are highly dependent on their membrane fatty acid composition which is readily influenced by dietary fatty acid intake. Male Lewis rats were fed semi-purified diets containing two levels of α-linolenic and linoleic acids for 70-80 days. Lymphocytes were isolated and the fatty acid composition of their major phosphoglycerides was determined. Lymphocyte function *in vitro* was assessed using the lymphocyte activation assay. To determine if altered lymphocyte function *in vitro* was reflected *in vivo* the incidence and severity of the inducible, autoimmune disease model—experimental allergic encephalomyelitis was assessed. This cell-mediated disease is characterized by lymphocyte infiltration into the central nervous system. Since prostaglandins (PG) are involved in the regulation of the immune response the production of $PGE_{1\&2}$ and $PGE_{2\alpha}$ by spleen, thymus and peritoneal macrophages was determined. Changed lymphocyte function, disease status and PG production will be discussed in terms of the altered fatty acid patterns of the lymphocytes and tissues induced by the different α-linolenic and linoleic acid dietary intakes and the possible function of these fatty acids in the immune response. This research was supported by the Science and Education Administration of the USDA under Grant #5901-0410-8-0061-0 from the Competitive Research Grants Office.

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WORLD FATS AND OILS SITUATION. E.H. Pryde, Northern Regional Research Center, 1815 North University, Peoria, IL 61604, and H.O. Doty, Economics, Statistics, and Cooperatives Service, USDA, Washington, D.C. 20250.

World production of edible fats and oils has grown by 27% over the last seven crop years—from 36 to almost 46 million metric tons. To this increase, the following vegetable oils have contributed the most (share of increase in percent): soybean (55), palm (20), rapeseed (9), and sunflower (9). While the world population has grown at the rate of 2% per year, vegetable oil production has grown at the rate of 3-4% per year, and these rates will likely continue with soybean, sunflower, and palm oils as the most important oils. At present, the six major sources of edible fats and oils are (share in percent): soybean (27), butter (13), sunflower (10), lard (9), palm (8), and rapeseed (7). Predominance of soybean oil can be ascribed to favorable agronomic characteristics of the soybean plant over a wide area in the temperate zones, to the coproduction of low-cost and high-quality protein useful to both humans and animals and to the development of refining procedures that produce a widely available and low-priced edible oil in large quantities. World production of inedible fats and oils has not kept pace with that for edible oils and has increased by only about 6% over the last seven crop years—from 6.6 to 7.1 million metric tons. In the U.S., edible vegetable oils contribute at least as much to industrial products as do the inedible vegetable oils. It may be that the world can and should rely more upon these renewable resources than it does now for manufacture of industrial goods in order to supplement other resources and to prepare for the time when petrochemicals become even less available and more costly. To ensure increased production of fats and oils, it is necessary to continue not only the development of new varieties with improved oil (and protein) yields but also the search for new and unconventional sources.

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MORPHINE-FREE POPPY AS A NEW SOURCE OF EDIBLE OIL. Östen Levin, Margarinbolaget AB, Fack, 104 25 Stockholm 30, Sweden, and Ulf Nyman, Svalöv Co. Ltd., Svalöv, Sweden.

In view of increasing opiate abuse high contents of morphine have been considered a disadvantage for the utilization of *Papaver somniferum* L. as an oil crop. Selection for low morphine content has resulted in the release of a variety, Sv. Soma, in which morphine and other alkaloids are present in contents of around 1/10 of that normally found. Another aim in the breeding work is to select for high contents of the non-narcotic alkaloid thebaine. This alkaloid can improve the economy of poppy cultivation as it can be used by the pharmaceutical industry similar to morphine. Some difficulties in cultivation technique, e.g. sowing of the small seeds and weed control, will also be discussed. Seeds from this blue-seeded cultivar contains over 40% of oil, which can be obtained by pressing and subsequent extraction of the press cake. The composition of the oil as well as refining characterities will be discussed. Feeding studies in rats indicate the oil has a high nutritional value.

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YEASTS AND MOLDS AS SOURCES OF OILS AND FATS. Colin Ratledge, Department of Biochemistry, University of Hull, HULL, HU6 7RX, England.

The case for using micro-organisms as sources of oils and fats will be considered against the current supply and demand of conventional plant and animal oils. The key factors may be seen as selection of an appropriate organism, development of the most efficient system for its growth and fat accumulation, and provision of an adequate and cheap supply of carbon substrate for the organism to metabolize. Microbial oils as substitutes for bulk commodities such as palm oil, groundnut oil or even cocoa butter can now be produced as well as ones having high contents of polyunsaturated fatty acids which would make them suitable for medicinal applications. Microbial oils suitable for a range of technical purposes are also feasible propositions. The talk will attempt a brief overview of the topic as well as a discussion of some of the work being carried out in the author's laboratory.

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OIL-RICH FOREST OILSEEDS. S.M. Osman and F. Ahmad, Department of Chemistry, Section of Oils & Fats, Aligarh Muslim University, Aligarh, India.

The PL-480 Project, undertaken to survey wild oilseed wealth of the Northern Indian flora, has revealed the potential of some little-known forest oilseeds. Screening of oils from more than 200 species has shown few oilseeds to be unusually rich in conventional or unusual fatty acids. These species are: *Ochna squarrosa,* ochnaceae family, (16:0, 70%); *Martynia diandra,* pedaliaceae, (16:0, 48%); *Justicia adhatoda,* acanthaceae, (18:1, 50.6%); *Xanthium strumarium,* compositae, (18:1, 54.3%); *Pogestemon plectranthoides,* labiatae, (18:2, 70%); *Carthamus oxycantha,* compositae, (18:2, 71%). Two species, *Chrozophora rottleri,* euphorbiacae, and *J. adhatoda* yielded protein-rich oils containing 44 and 55% crude protein respectively. Oils of *J. adhatoda* and *X. strumarium* resemble groundnut oil in composition whereas *C. oxycantha* oil is fairly similar to safflower oil. Two new *Vernonia* sps. *V. roxburghii* and *V. valkemenifolia* were found to be epoxy acid-rich (63.5-74%) oils. In *V. roxburghii* the major epoxy acid vernolic (57%) is accompanied by a hitherto unknown epoxy acid (17.4%) characterized as *cis*-3,4-epoxy-*cis*-11-octadecenoic. Seed oil of *Wrightia coccinea,* apocyanaceae, has been found to be the richest known source of *iso*-ricinoleic acid (76%). Oil of an additional species of the genus *Iberis, I. odourata* is very interesting in having 20:1 acid (Δ^{11}-eicosenoic) as a major component but devoid of erucic acid. This oil is thus a typical zero-erucic oil of Cruciferae family. Seed oil of *Leucas aurticaefolia,* labiatae, was found to be a rich source of an allenic acid (Laballenic, 24%). The compositional data of the oils from the above species show sufficient promise for developmental research to find new sources of vegetable oils. These species, if found agronomically superior, are potential substitutes for the commercial oils now in use in edible and non-edible industries.

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POTENTIAL NEW SOURCES OF OIL FROM TROPICAL AMERICA. Richard Evans Schultes, Botanical Museum, Harvard University, Oxford Street, Cambridge, MA 02318.

The Amazon Valley and other parts of tropical America can be tapped for new sources of edible and industrial oils. There are literally several scores of plants rich in oils but exploited only by primitive societies and on a very primitive level from wild plants. Some of these trees deserve attention as possible new domesticated crops, but their biology in the wild must first be evaluated. Several prom-

ise, once domesticated, to be rivals of the rubber tree in importance as cultivated crops. A number of these potential new cultigens will be discussed.

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THE JESSENIA PALM. Michael J. Balick, Botanical Museum of Harvard University, Oxford St., Cambridge, MA 02138.

The *Oenocarpus-Jessenia* complex of Amazonian palms produces an edible oil closely resembling olive oil both chemically and physically, in taste. *Jessenia bataua* is the most widespread species, growing throughout the Amazon Valley on either dry or swampy land. Indians have always known about the uses of this palm; in preconquest times, some tribes based part of their trading economies on its exploitation. The palms in this complex represent a potential crop for tropical nations; one that yields a high quality oil with strong consumer demand. The fruit can be made into a beverage or oil, and pulp obtained as a byproduct for use as an animal feed. Investigation has shown the nutritional quality of these products to be strikingly high. Further efforts towards the goal of domesticating this species are being made.

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FATS AND OILS – A REGULATORY AND NUTRITIONAL UPDATE. Victor Frattali, Department of Health, Education, and Welfare, Public Health Service, Food and Drug Administration, Washington, DC 20204.

In contrast to years of neglect, nutrition has begun to receive a rapid increase in attention and interest from the highest national arena down to the level of the consumer. Over the past few years, for example, intense discussions have focused on the development of "dietary goals" by a congressional committee in an attempt to foster progress in the prevention of leading chronic diseases such as coronary artery disease and obesity. Of interest is the recommendation for a reduction in total fat consumption from about 40 percent to about 30 percent of energy intake. In addition, it is recommended that saturated fat intake be reduced to account for about one-third of the total fat consumption, with monounsaturated and polyunsaturated fat intake contributing equally to make up the balance. Recently, epidemiological data have been interpreted by some as suggesting that high fat intake is associated with an increased risk of cancer, thus providing impetus for the development of national dietary guidelines. This heightened interest and activity has had an impact on regulatory issues in nutrition and food labeling policy, particularly nutrient labeling. Consequences of this impact will be discussed along with other regulatory aspects dealing with fats and oils.

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ALGAE AS SOURCES FOR EDIBLE LIPIDS. R.G. Ackman, Nova Scotia Technical College, P.O. Box 1000, Halifax, N.S. B3J 2X4, Canada.

Algae include some freshwater and many marine species traditionally harvested for food purposes. In the future algae could be cultured specifically to meet nutritional demands for selected fatty acids such as γ-linolenic (18:3ω6) or eicosapentaenoic (20:5ω3) acids, or for rare or unusual unsaponifiable materials such as hydrocarbons, sterols etc. Algae primarily being harvested for industrial polysaccharides will be reviewed in terms of raw materials for recovery of fatty acids and lipids or of proteins. A wide variety of seaweeds have recently been placed into culture on both experimental and commercial bases. The yields can be of the order of 100 tons of dry weight per hectare per year. Genetic manipulation of seaweeds is just beginning and can produce superior yields or alter chemical compositions in desired directions. Compared to yeasts and molds, which have been intensively investigated as sources of single-cell protein, seaweeds and unicellular algae have received little attention. They do not require specific carbon sources and the effect of the latter on fatty acid details, such as the odd-chain fatty acids found in yeasts grown on alkane mixtures, is absent.

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OKRA. Franklin W. Martin, Small Farm Prod. Systems Project, USAID/ROCAP, CATIE, Turrialba, Costa Rica, and Lehe Telek,* USDA/SEA/AR/SR, Mayaguez Institute of Tropical Agriculture, Box 70, Mayaguez, Puerto Rico.

The potential of dry seed of okra (*Abelmoschus esculenta*, Moench) as high protein and high oil crop have been investigated from 1977-79 in Mayaguez, Puerto Rico. A world collection of 238 varieties has been planted and seeds were chemically evaluated for oil and protein. Oil yield varied from 17-22% and on a per hectare basis the yield was higher than that of soybean, varying from 289 to 612 kg/ha. Yields of protein were seen to compete favorably with protein yields of other oil crops. A large percentage (61%) of the fatty acids of okra seeds are unsaturated. The high level (41%) of linoleic acid, an essential fatty acid in human nutrition, makes okra a desirable oil seed crop. The equipment used to process cotton seed can be used without change to process okra seed.

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^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF OILS CONTAINING CONJUGATED TRIENOIC ACIDS. A.P. Tulloch and L. Bergter, Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan, Canada S7N OW9.

Naturally occurring conjugated trienoic acids have characteristic ^{13}C NMR spectra which can be used for identification and estimation. The chemical shifts of the 6 double bond carbons and of the 6 carbons adjacent (3 on each side) to the triene system indicate the positions and configurations of the double bonds. Mixtures of conjugated trienoic acids in seed oils have not been readily analyzed but the relative amounts of these acids can be measured by ^{13}C NMR. The composition of oil from *Fevillea trilobata* has been determined in this way. ^{13}C NMR spectra of other oils containing conjugated trienoic acids and of products of isomerization of nonconjugated trienoic acids will be discussed.

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^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF THE FOUR METHYL 12, 15-OCTADECADIENOATE GEOMETRIC ISOMERS. Henry Rakoff, David Weisleder* and Edward A. Emken, Northern Regional Research Center, AR, SEA, USDA, 1815 North University, Peoria, IL 61604.

^{13}C NMR chemical shifts were determined for all the carbon atoms of methyl *cis, cis-, cis, trans-, trans,cis-* and *trans, trans*-12, 15-octadecadienoate and were used to confirm the identity of these compounds. These experimental values are compared with theoretical values calculated from published chemical shift parameters (Chem. Phys. Lipids *17*, 501 (1976); *18*, 115 (1977)). Chemical shift parameters have been determined that permit calculation of chemical shifts for certain carbon atoms in compounds where a *trans* double bond is near the methyl end of a carbon chain.

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^{13}CMR OF EPOXY FATTY ACIDS. David Weisleder, Northern Regional Research Center, AR/SEA, USDA, 1815 N. University St., Peoria, IL 61604.

Carbon-13 magnetic resonance spectra have been obtained for a number of fatty acids containing either a *cis* or *trans* epoxide group (or moiety). The chemical shift of the methylene carbon adjacent to an epoxide exhibits a large dependence on the stereochemistry of the epoxide similar to that found for carbons allylic to olefins. The difference in chemical shift between the epoxy carbons increases significantly when the epoxide is adjacent to a double bond. The position as well as the configuration of the epoxy group in octadecenoic acids can be established by ^{13}CMR.

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NUCLEAR MAGNETIC RESONANCE STUDIES ON THE MYCOLIC ACIDS. D.E. Minnikin and S.M. Minnikin, Department of Organic Chemistry, The University, Newcastle upon Tyne, NE1 7RU, Great Britain.

Mycolic acids are high molecular weight long-chain 2-branched, 3-hydroxy acids first isolated from mycobacteria but also occurring in related bacterial genera. Mycobacterial mycolates are the largest ($C_{60} - C_{90}$) and most complicated in structure including components having oxygen functions(\C = 0, \CH – OCH_3, –COOH) in addition to the 3-hydroxy acid unit. In contrast the mycolic acids of corynebacteria, rhodococci and nocardiae are relatively simple mixtures of olefinic (0-5 double bonds) molecules ranging in size from C_{20} to C_{70} depending on the source. The carbon skeletons of mycobacterial mycolates also more complicated in that they may include *cis* or *trans* double bonds and cyclopropane rings and methyl branches. Nuclear magnetic resonance (NMR) spectroscopy is the procedure of choice for the characterization of the functional groups present in mycolic acids but proton NMR determinations are hampered by the overwhelming presence of a large number of aliphatic methylene groups. The greatly increased chemical shift differences observed in ^{13}C NMR spectra allow much clearer spectra to be obtained for long-chain compounds though large amounts (>100 mg) are desirable for analysis. The present communication will show that ^{13}C NMR allows the clear observation of the resonances produced by the functional groups present in mycolic acids and their derivatives. In particular *erythro* and *threo* isomers of the 3-hydroxy acid unit are readily distinguished.

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^{19}F NMR OF FLUORINE LABELED FATTY ACIDS AND PHOSPHOLIPIDS IN LIPID BILAYER VESICLES. James R. Cavanaugh, Philip E. Pfeffer and Kathleen M. Valentine, Eastern Regional Research Center, Agricultural Research, SEA, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Fluorine labeled long-chain fatty acids were incorporated into phospholipid vesicles prepared by sonication of aqueous lipid emulsions. Characterization by gel permeation chromatography showed that stable vesicles could be obtained with L-α-dimyristoylphosphatidylcholine (DMPC) provided the fatty acid concentration did not

exceed 5% by weight of lipid. Vesicles containing 4,4-difluorostearic acid, 6,6-difluoromyristic acid, 12,12-difluorostearic acid, or ω-fluoropalmitic acid gave rise to single, narrow ^{19}F resonances with line widths at half height of approximately 10 Hz at 70C. When cholesterol was incorporated into the DMPC-fatty acid vesicles, the fluorine resonance showed a corresponding increase in broadening. The line widths also showed a systematic increase for the 40% cholesterol samples as the fluorine substitution advanced up the chain. Vesicles were also prepared from the fluorine labeled phospholipids, L-α-*bis* (12,12-difluorosteroyl)-phosphatidylcholine and L-α-*bis*-(ω-fluoropalmitoyl)-phosphatidylcholine, both individually and incorporated into host vesicles. These vesicles gave rise to two ^{19}F resonances as observed previously. The extent of the separation depended on both the particular phospholipid labeled and the host phospholipid. The nature of the chain-chain interaction appears to differ significantly in the labeled fatty acid vesicle compared to that in the corresponding labeled phospholipid.

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STUDIES OF CHLOROPHYLL *A* IN MODEL AND NATURAL MEMBRANE SYSTEMS. K.E. Eigenberg, W. R. Croasmun, and S.I. Chan, A.A. Noyes Laboratory of Chemical Physics 127–72, California Institute of Technology, Pasadena, CA 91125.

A variety of evidence suggests that lipids of the photosynthetic thylakoid membrane have, to some extent, a role in photosynthesis. We have recently been concerned with the hypothesis that chlorophyll *a* interacts with membrane lipids to form an antenna array on the thylakoid surface. In order to better understand the properties of chlorophyll incorporated into a bilayer matrix, we have studied the chlorophyll *a*/distearoyllecithin (DSL) bilayer membrane system. The phase diagram of the system indicates compound formation between chl *a* and DSL. Magnetic anisotropy of the chlorophyll ring system causes large ring current shifts of nuclei on nearby molecules depending on their distance and orientation with respect to the porphyrin plane. We have used this effect to verify by ^{31}P NMR the existence of two distinct phases of phospholipid below the solidus as predicted by the phase diagram. ^{13}C NMR in conjunction with the ^{31}P result shows that the interaction between chl *a* and DSL occurs via coordination of the chl *a* central Mg atom to the phosphate moiety of DSL. ^{1}H spectra as a function of temperature show shifts of DSL headgroup resonances which facilitate interpretation of the phase diagram. High resolution ^{13}C spectra at 90.5 MHz have been obtained from thylakoid membranes. These show resonances attributable to the sugar headgroups of monogalactosyldiglyceride as well as the typical 18:3 linolenic acid chains. Protein and chlorophyll headgroup resonances are absent, presumably due to motional restriction. Phytol resonances from chlorophyll are also observed. This indicates that the motional state of the phytol chain is not governed by protein, a fact which may be consistent with protein-independent chlorophyll.

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NMR AND MASS SPECTROMETRY OF ANACARDIC ACID DERIVATIVES. Gayland F. Spencer, Northern Regional Research Center, AR/SEA, USDA, 1815 N. University St., Peoria, IL 61604.

Anacardic (6-alkylsalicylic) acids are known to occur naturally in oils from certain Anacardiaceae and Gingkoaceae. Recent work has disclosed a new source of these acids, *Knema elegans* (Myristicaceae) seed oil, which also contains acids that have the alkyl chain terminated by a phenyl rather than a methyl group. Characterization of anacardic acids is facilitated by nuclear magnetic resonance and mass spectrometry of suitable derivatives. Spectra discussed include the trimethylsilyloxy-trimethylsilyl esters 2nd the acetoxy-methyl esters of both alkyl and phenylalkyl anacardic acids.

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FIELD DESORPTION MASS SPECTROMETRY AS A TOOL FOR THE STRUCTURE DETERMINATION OF LIPIDS AND RELATED COMPOUNDS. Catherine E. Costello, Department of Chemistry, 56-029, M.I.T., Cambridge, MA 02139.

Field desorption extends the range of compounds which may be analyzed by mass spectrometry to include salts and other energy-labile materials. Like many molecules of biological interest, fats and other polar lipids have presented some difficulty to the mass spectrometrist. Techniques requiring volatilization of the sample before ionization (electron impact, chemical ionization) are frequently not suitable for these compounds because they are subject to pyrolysis or undergo excessive fragmentation resulting from the amount of energy transferred during the ionization process. Desorption of the sample from a specially prepared emitter under the influence of a high electric field, however, results in a minimal energy transfer and the field desorption (FD) spectra obtained in this manner usually include abundant molecular ions and few fragments. It is thus possible to determine molecular weights and to deduce structural details of these compounds, even without derivatization. It has been found, moreover, that the rate of desorption may be more carefully controlled and some informative fragmentation obtained if certain types of derivatives are prepared and analyzed as well. When this technique is used in combination with other types of mass spectrometry and with other analytical methods, such as nuclear magnetic resonance, quite complex structures can be determined. Examples to be discussed will include studies involving lecithins, glycolipids, other related molecules and biological membranes.

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^{1}H AND ^{13}CMR SPECTRAL STUDIES OF FURANOID FATTY ACIDS. M.S.F.Lie Ken Jie, Chemistry Department, University of Hongkong, Hong Kong.

The proton magnetic resonance spectra of all non-methyl substituted synthetic positional isomers of C_{18} furanoid fatty acids will be discussed, revealing the effect of shift reagents in the identification of positional isomers. The ^{13}CMR spectral behavior of the same series of fatty acids show differentiation in the carbon shifts for most positional isomers with certain limitations.

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EVIDENCE FOR THE FORMATION OF A SPECIFIC α-TOCOPHEROL-LIPOXYGENASE COMPLEX. Shlomo Grossman, Eugenia G. Waksman, and Ira S. Hammerman, Department of Natural Sciences Bar-Ilan University, Ramat-Gan, Israel.

Solutions of ^{3}H-α-tocopherol (Vitamin E) when percolated through soybean lipoxygenase-sepharose columns exhibit pronounced binding of the vitamin to the immobilized enzyme. The formation of the α-tocopherol-lipoxygenase complex was studied both with immobilized commercial soybean lipoxygenase and immobilized affinity purified enzyme. The molar ratio of the α-tocopherol-lipoxygenase complex was dependent on the concentration of the reactants. The binding of one molecule of α-tocopheral per enzyme molecule resulted in 50% inhibition of linoleate oxidation by the enzyme. The α-tocopherol-lipoxygenase complex did not dissociate on addition of linoleic acid, the classical substrate for lipoxygenase. When sepharose-bound lipoxygenase was treated with iodoacetate at acidic pH, the enzyme failed to form the complex with α-tocopherol. As previously shown, iodoacetate modifies an essential methionine residue in lipoxygenase. Further evidence for the specificity of the α-tocopherol-lipoxygenase complex came from an affinity chromatography experiment in which lipoxygenase was chromatographed on an aminoethyl linoleyl sepharose column and the bound enzyme was eluted with α-tocopherol. This eluate exhibited very low specific activity in linoleate oxidation. Digestion of the ^{3}H-α-tocopherol-lipoxygenase complex with proteolytic enzymes followed by gel filtration on sephadex G-25 column resulted in the separation of several peptides with most of the radioactivity incorporated into one peptide only. Analysis of the isolated peptide is now under progress for determination of the α-tocopherol binding site in the enzyme molecule.

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SOME PROPERTIES OF THE TYPE-2 LIPOXYGENASE FROM SOY BEANS. W. Grosch, P. Schieberle, and R. Kieffer, Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-8046 Garching, West Germany.

Purified type-2 lipoxygenase was incubated with linoleic acid, methyl linoleate and Ca^{2+}-linoleate at pH 6.5. The effects of enzyme, substrate and detergent concentrations and of temperature were studied. In all experiments the enzyme forms a wide range of products. Monohydroperoxides (5–15 mol-% of consumed linoleic acid) and oxooctadecadienoic acids (5–10 mol-%) were identified and analyzed with respect to regio- and stereo-specifity in the oxygenation reaction. From the results it was concluded that the type-2 lipoxygenase acts as an autoxidation catalyst which forms free radicals. Further experiments show that this property of the enzyme is responsible for the co-oxidation of carotenoids and for the action of soybean flour in the baking process.

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LIPID OXIDATION AND FLAVOR BIOGENESIS IN EDIBLE PLANTS. Terry Galliard, R.H.M. Research Ltd., The Lord Rank Research Centre, Lincoln Road, High Wycombe, Bucks. HP12 3QR U.K.

The initial process in the formation of volatile flavor compounds from fatty acids is usually oxidation to hydroperoxide. In many plants, this reaction is catalyzed by lipoxygenase enzymes and the resultant fatty acid hydroperoxides are degraded by enzymic or non-enzymic processes to release volatile derivatives. In most food applications, the volatile compounds are undesirable and represent off-flavor etc. However, in some fruits and vegetables, these compounds are necessary for the desirable and characteristic flavor attributes. This paper will present the major conclusions from a recently complete research program in the author's previous laboratory at the A.R.C. Food Research Institute, Norwich, in which a detailed study was made of the biogenesis of volatile aldehydes in cucumber and tomato fruits. C_9-unsaturated aldehydes are responsible for the characteristic flavor of cucumber and C_6-aldehydes

contribute to the flavor of fresh tomato fruits. These aldehydes are not present in the intact tissue but are formed immediately on disruption by cutting, etc. In both materials, 9-hydroperoxides of linoleic and linolenic acids are produced by the action of lipoxygenase. However, subsequent conversions appear to be selectively controlled by specific enzyme action in cleaving the hydroperoxides. Details of the proposed mechanisms will be discussed.

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INTERACTIONS BETWEEN PLANT LIPOXYGENASE SYSTEMS AND ESSENTIAL FATTY ACIDS. E.V. Boudnitskaya and I.G. Borisova, Institute of Biochemistry, Academy of Sciences USSR, Moscow, U.S.S.R.

The isoenzymes composition and kinetic parameters of the lipoxygenases of seeds and meristems of seedlings roots of plants were studied. It is shown that the composition of the molecular forms of lipoxygenase systems is different. Enzyme-catalyzed oxidation of essential fatty acids in vitro differ in the kinetic parameters, structure of intermediates of fatty acids oxidation and amount of the final products. The lipoxygenase plant systems have differing ability to form oxidation substrates including those with singlet oxygen. The highest catalytic activity is found in heterogenous lipoxygenase *(Pisum sativum* and *Glycine hispida)* with 3–5 molecular forms. The lipoxygenase *(Vicia faba* and *Faseolus vulgaris)* with 2–3 molecular forms show a low enzyme activity and reduced ability of oxidizing linoleic and arachidonic acids. Any of the molecular forms separated shows a lower catalytic activity and the kinetics of essential fatty acids oxidation change. The experimental data obtained allow a conclusion that the mechanism of heterogenous lipoxygenase systems and essential fatty acids reactions depends on the composition of the molecular forms of plant lipoxygenases.

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PURIFICATION AND SOME PROPERTIES OF WHEAT GERM LIPOXYGENASE. Jacques Nicolas, Maria Autran, and Roger Drapron, Laboratoire de Technologie Alimentaire de l'I.N.R.A. au CERDIA, 91305 Massy, France.

The purification of lipoxygenase to almost homogeneity has been obtained from wheat germ by a classical scheme. After extraction at pH 4.5 from defatted germ, lipoxygenase activity was precipitated by 40% saturation $(NH_4)_2SO_4$ from 25% saturation supernatant. After dissolution in a phosphate buffer at pH 7 and dialysis against this same buffer, the extract was submitted to gel filtration on Ultrogel AcA34. The final step of DEAE Sephadex A50 chromatography has given three lipoxygenase fractions. The total yield of the purification process is close to 30% and the degree of purification varies from 200 to 300 depending on the fraction which is considered. The three isoenzymes were also detected by disc-electrophoresis with a specific staining of lipoxygenase activity and by isoelectrofocussing in a liquid medium. Some properties, such as molecular weight and isoelectric points, have been determined for each fraction. pH optima, thermal treatment effect and β-carotene cooxidizing power have been studied.

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LIPOXYGENASE INHIBITION BY NATURALLY OCCURRING FATTY ACIDS. Robert L. Ory and Allen J. St. Angelo, USDA, SEA, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Lipoxygenase is high in soybeans, with lesser amounts in peanuts and no detectable activity in several varieties of rapeseed. For an explanation of the lack of activity in rapeseed, several fractions were analyzed for lipoxygenase activity and possible inhibitors. Erucic acid inhibited both soybean and peanut lipoxygenases. Various long-chain monounsaturated fatty acids were assayed for their effects on lipoxygenase activity with linoleic acid as substrate. Fatty acid chain length (C-16 to C-24) was not a significant factor, but position of the double bond and *cis/trans* isomers did affect enzyme activity. The position of the double bond from the methyl terminal end seems to be more significant than distance from the carboyxl end in terms of inhibition.

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NEW ASPECTS OF THE INHIBITION OF LIPOXYGENASE. J. Verhagen and J.F.G. Vliegenthart, Department of Bio-organic Chemistry, State University Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands.

Lipoxygenase [EC 1.13.11.12] catalyzes the conversion of polyunsaturated fatty acids containing a 1,4 *cis,cis*-pentadiene system into conjugated hydroperoxides. Inhibition of the oxygenating activity of the enzyme can be achieved along several routes. Substrate analogs of substrate fatty acids like conjugated linoleic acid are well-known efficient inhibitors. Chemical modification of the polypeptide chain in or close to the active site may lead to reduction of the enzymic activity or to inactivation. Interestingly, specific modification of 3 of the 5 sulfhydryl groups of soybean lipoxygenase with CH_3HgI gives rise to a modification of the activity pattern. The properties of the modified enzyme are very similar to those of the type 2 enzymes: *(i)* a considerable substrate inhibition can be observed at high pH (9–10), *(ii)*. The regio- and stereospecificities of the oxygenation are significantly lower than for the native enzyme, *(iii)* besides hydroperoxides also oxodienoic acids are formed as products (is 5%), and *(iiii)* enhanced capacity for the oxidation of cosubstrates. Inhibition of the enzymic oxygenation of substrate fatty acids can also be obtained by various antioxidants. The way of action of these compounds is usually ascribed to the antioxidative properties. However, since iron has been demonstrated to occur in lipoxygenase and to play an essential role in the aerobic and anaerobic reactions catalyzed by this enzyme, it cannot be excluded that these compounds coordinate to irons, thereby giving inhibition. For 4-nitro catechol and some other 0-diphenolic compounds it could be shown that these are capable of forming a 1:1 complex with lipoxygenase, provided that the enzyme occurs in the Fe III state. The inhibition of the enzyme by 4-nitro catechol is of the pseudo non competitive type.

Hydrogen peroxide is a very powerful reagent to inactivate lipoxygenase irreversibly. This compound reacts with iron in the native enzyme, which is converted into a high spin ferric species. Although several physical parameters of the H_2O_2-treated enzyme are identical to those of the enzyme which has been converted with one equivalent of product hydroperoxide, it was found that CD spectroscopy is suitable to distinguish between these enzyme species.

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SPECIFICITY OF TYPE-2 LIPOXYGENASES. G.A. Veldink, L.J.M. Spaapen, C.P.A. van Os, and J.F.G. Vliegenthart, Department of Bio-organic Chemistry, State University Utrecht, Groesestraat 79 3522 AD Utrecht, The Netherlands.

Lipoxygenases (linoleate: oxygen oxido-reductase, EC 1.13.11.12) can be classified according to a number of physical and chemical properties into two main categories: the type-1 group of enzymes which comprises the well-studied soybean lipoxygenase-1, and the type-2 group which contains enzymes from a wide variety of plant sources, including soybeans. One important parameter in distinguishing the two major classes of lipoxygenases has been their pH-optimum. The type-1 lipoxygenase has an optimum at pH 9–10 whereas lipoxygenase-2 is generally considered to have its optimum at pH 6–7. However, recent experiments have revealed that the regio- and stereospecificities in the oxygenation of linoleic acid by lipoxygenase-2 are considerably enhanced by raising the pH of the reaction medium to 9. On the other hand, chemical modification of soybean lipoxygenase-1 with CH_3HgI generates an enzyme species with a number of catalytic properties reminiscent of lipoxygenase-2 viz. Partial loss of regio- and stereospecificities at lower pH and a relatively strong co-oxidizing capacity.

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CHARACTERISTICS OF PURIFIED LIPOXYGENASE ISOENZYMES. Barbara P. Klein, Department of Foods and Nutrition, 274 Bevier Hall, University of Illinois, Urbana, IL 61801.

The existence of multiple forms of plant lipoxygenase is well established in soybeans, and may occur in other vegetables and seeds as well. Soybeans have at least three isoenzymes which have been extensively studied. Three lipoxygenase isoenzymes have been purified and characterized from pea seeds. A fourth pea isoenzyme may exist in extremely small quantities. Substrate specificity, pH profiles, molecular weight, isoelectric point, carotene and chlorophyll bleaching have been investigated. The three major purified pea isoenzymes can be differentiated from one another on the basis of those parameters, and appear similar in their behavior to the multiple forms of soy lipoxygenase. Characteristics of other plant lipoxygenases are varied, and they have not been as extensively purified. However, there is evidence that similar lipoxygenase isoenzymes can be found in different plant materials as well as in different varieties of the same seed.

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SPECIFICITY OF HYDRAZINE REDUCTION OF ETHYLENIC BONDS AS AN AID TO THE IDENTIFICATION OF FATTY ACIDS. W.N. Ratnayake and J.S. Grossert, Dalhousie University, Halifax, and R.G. Ackman, Fisheries Research and Technology Laboratory, Nova Scotia Technical College P.O. Box 1000, Halifax, Nova Scotia B3J 2X4.

Polyunsaturated fatty acids (PUFA) were partially reduced with hydrazine in ethanol at 40 C. The reduction products have been characterized by open-tubular gas liquid chromatography, argentation thin layer chromatography and by oxidative ozonolysis with BF_3-MEOH. Partial hydrogenation of all-cis-6,9,12,15-hexadecatetraeonic and all-cis-3,6,9,12,15-octadecapentaenoic acids indicated that reduction occurs most rapidly at the terminal and proximal ethylenic bonds. In the early stages of hydrogenation of methylene-interrupted PUFA, the proportions of products reflected the reac-

tivity of the individual ethylenic bonds. The proportions of the different isomeric monoethylenic fatty acids formed show a Gaussian distribution with the maxima at the center of the original ethylenic system and among the reduction products the nonmethylene-interrupted isomers predominated within each group of dienes, trienes, etc. These results are explained as due to slow reactivity of nonmethylene-interrupted counterparts. After about 30 minutes of reduction of 9,12,15-octadecatrienoic acid, the relative proportions of monoethylenic fatty acids were 18:1Δ12>18:1Δ9>18:1Δ15 and the major diethylenic isomer was 18:2Δ9,15. The major factors contributing to the relative amounts of monoethylenic fatty acids formed during partial hydrogenation of PUFA will be discussed in terms of the number, position and configuration of the ethylenic bonds. The procedure will be illustrated by the application of hydrazine reduction, coupled with argentation TLC and oxidative ozonolysis, to the identification of 9,12,15-hexadecatrienoic, 8,11,14- and 11,14,17-octadecatrienoic, and 8,11,14,17-octadecatetraenoic acids from the seaweed *Agarum cribrosum*.

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OXIDATION OF UNSATURATED AND HYDROXY FATTY ACIDS BY RUTHENIUM TETROXIDE AND RUTHENIUM OXYANIONS. Y. Nakano and T.A. Foglia, Eastern Regional Research Center, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, PA 19118.

The reactions of ruthenium VIII tetroxide (RuO_4) and the ruthenium VII and VI oxyanions, perruthenate (RuO_4^-) and ruthenate ($RuO_4^=$) with hydroxy substituted fatty acids and unsaturated fatty acids have been investigated. At a 1:1 molar ratio, ruthenium tetroxide (RuO_4) and both oxyanions (RuO_4^- and $RuO_4^=$) cleanly and selectively oxidized 12-hydroxystearic acid to 12-ketostearic acid. With the vicinal dihydroxy compound 9,10-dihydroxystearic acid the type of oxidation products obtained depended on the amount of ruthenium oxidant employed. At high ratios of oxidant to substrate, cleavage to pelargonic and azelaic acids occurred, whereas at lower ratios partial oxidation to diketo and acyloin products was observed. The oxidation of oleic acid with either excess ruthenium tetroxide (RuO_4) or perruthenate (RuO_4^-) also gave the cleavage products pelargonic and azelaic acid. Cleavage of the double bond occurred through the step-wise information of the dihydroxy and kiketo intermediates. In contrast, ruthenate ($RuO_4^=$) did not react with the double bond of oleic acid. The mechanistic implications of these studies on the pathway of ruthenium oxide oxidations will be discussed.

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REACTION OF SINGLET OXYGEN WITH UNSATURATED FATTY ACIDS ADSORBED ON METAL OXIDE SURFACES. Y. Nakano, T.A. Foglia, and D.A. Konen, Eastern Regional Research Center, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, PA 19118.

The reaction of singlet oxygen with unsaturated fatty acids either in solution or adsorbed on metal oxide surfaces has been investigated. The singlet oxygen was generated either chemically or photochemically with a photosensitizer. In solution, singlet oxygen reacted with the double bond of the fatty acid to yield an allylic hydroperoxide. When the unsaturated fatty acid was adsorbed onto the surface of a metal oxide, however, reaction with singlet oxygen gave rise to cleavage products as well as hydroperoxides. The yield of cleavage products was dependent on the type of metal oxide used. For example, the reaction of oleic acid adsorbed onto an Al_2O_3 surface gave equal amounts of double bond cleavage products and allylic hydroperoxides. On the other hand, only small amounts of cleavage products were found when the oleic acid was adsorbed on CaO or MgO. We believe that formation of double bond proceeds through the formation of a dioxethane intermediate. The role of the metal oxide surfaces in promoting this cleavage reaction will be discussed.

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CARBOXYLIC ACIDS FROM THE CAUSTIC OXIDATION OF ISOTRIDECYL OXO BOTTOMS. N.E. Lawson, Research & Development, Union Camp Corporation, P.O. Box 412, Princeton, NJ 08540, and M.J. Harding, FMC Research.

Caustic oxidation has been utilized to convert the complex residue from the isotridecyl alcohol process ("C_{13} oxo bottoms") to simpler mixtures of isotridecanoic and higher boiling carboxylic acids. The predicted and actual products are discussed relative to the composition of the oxo bottoms. They are highly branched primary and secondary fatty acids which have potential commercial value because of their liquidity, stability, oil solubility and low costs.

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REACTION VARIABLES AFFECTING THE THERMAL ALTERATION OF METHYL OLEATE IN THE PRESENCE OF MINERAL CATALYSTS. T.A. Foglia and T. Perlstein, Eastern Regional Research Center, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, PA 19118.

The thermal treatment of methyl oleate in the presence of a mineral catalyst results in a number of concurrent reactions, including: elaidinization, isomerization (double bond migration), cracking (chain cleavage), chain branching (isoacids) and polymerization (dimer acids). For example, when methyl oleate was heated with 5% clay catalyst in an autoclave under N_2 atmosphere at 250 C for 24 hours the following reaction product mixture was obtained (by weight percent): elaidinization and isomerization 22.2%, cracking 8.8%, chain branching 37.4%, dimerization 21.6%. A number of reaction parameters were found to affect the course and extent of these concurrent reactions. Reaction variables studied included temperature, time, atmosphere, catalyst concentration, the use of co-catalysts and various types of catalysts. Greatest changes in the product composition were obtained when a co-catalyst was used in conjunction with the clay catalyst. For example, when dichlorethylene ($ClCH_2CH_2Cl$) was used as the co-catalyst the amount of the branched-chain esters in the product mixture increased to ≈60%, whereas the dimer products decreased to ≈30%. When isobutylene was used as the co-catalyst, extensive alkylation of the C_{18} chain occurred, giving rise to C_{22} to C_{30} branched alkyl esters.

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SYNTHESIS OF HYDROXY FATTY ACIDS. Suzanne R. Macaulay, Prairie Regional Laboratory, National Research Council of Canada Saskatoon, Saskatchewan, S7N OW9, Canada.

Long chain α,ω-diols and ω-hydroxy acids occur in both plant and animal waxes. A general synthesis of these compounds has been developed; for example, 1-24-tetracosanediol and 24-hydroxytetracosanoic acid have been prepared. The method is based upon the isomerization of internal triple bonds of alkyn-1-ols to alkyn-1-ols with terminal triple bonds. A new reagent which has been developed for this reaction will be discussed.

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PREPARATION OF THE *CIS-TRANS* ISOMERS OF LINOLEIC ACID. Maurice Naudet and Jean-Luc Perrin, Laboratoire National des Matieres Grasses-Iterg, Université d'Aix-Marseille–Place V. Hugo–F13331 Marseille Cedex 3 (France).

The *cis trans* isomers of linoleic acid (mixture of 9c 12t and 9t 12c octadecatrienoic acids) have been obtained in a high grade of purity according the following scheme. High purity linoleic acid (96.5%) is obtained from grapeseed oils fatty acids through inclusion in urea, then submitted to the isomerizing action of nitrous oxides during a short period of time at room temperature. After chromatographic purification, repeated crystallizations from acetonic solutions at low temperature lead to an 85% pure product. Final purification leading to a product of more than 98.5% purity is realized, after methylation, by complexation chromatography on a cation exchange resin saturated with silver ions.

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HYDROGENATION OF CARBOXYLIC ACIDS WITH SYNERGISTIC CATALYSTS. Bhupendra C. Trivedi, Dace Grote, and Thomas O. Mason, Ashland Chemical Company, P.O. Box 2219, Columbus, OH 43216.

Rhenium heptoxide, a known catalyst for hydrogenation of carboxylic acids to alcohols, forms synergistic combinations with palladium, platinum, rhodium and ruthenium catalysts. This effect is also seen at lower pressures (500 psi). Synergism is also manifest when rhenium and palladium (or rhodium) are used as supported catalysts on silica and used in a flow mode. An interaction of unknown nature between the metals suggests itself. The process is not very efficient at lower pressures, giving lower conversion in the flow mode. At higher temperatures needed for higher rates, significant participation of side reactions such as decarboxylation of the acid and hydrogenolysis of the alcohol occurs yielding hydrocarbons.

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THE FATE OF FATTY CYCLOPROPENOIDS IN THE PRESENCE OF THE LEWIS ACID BORON TRIFLUORIDE. Ian S. Gilkison and Geoffrey G. Shone, School of Chemical & Physical Sciences, Kingston Polytechnic, Kingston-upon-Thames, Surrey, KT1 2EE, United Kingdom.

As a preliminary to the study of the fate of fatty cyclopropenoids in the presence of acidic earths under refining conditions, we have investigated the nature of the products formed by treating methyl sterculate with the non-protonating acid boron trifluoride (as etherate), where no complications were introduced by varying adsorption properties of the acidic reagent. The complex mixture of products was separated using a combination of chromatographic methods, and structures were assigned on the basis of chromatographic, oxidation, reduction, and spectroscopic data. Entirely ring-opened products are formed during this reaction, a C_{18} alkyne and two isomeric C_{19} allenes being the major products. Other identified products include *cis-cis*, *cis-trans*, and *trans-trans* C_{19}

non-conjugated dienes; isomeric C_{20}-conjugated dienes with a methylene group in the 9/11 position; isomeric C_{19} ethers (9/10 ethoxyl-10/9 methyl-); and C_{38} and C_{39} monocyclic diesters of the Diels-Alder type. Trimers and polar dimers have also been found. The changing pattern of product composition with changing experimental conditions has been examined in order to establish the relationship between primary and secondary reaction products.

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HYDROPHOBIC INTERACTIONS OF PROTEINS WITH TRIGLYCERIDES. Lloyd M. Smith, Paolo Fantozzi, Richard K. Creveling and Robert E. Feeney, Department of Food Science and Technology, 3450 Chemistry Annex, University of California, Davis, CA 95616.

Interactions between proteins and lipids represent a major field of biological and technological interest. Hydrophobic interactions are difficult to study because of the low chemical reactivity of nonpolar groups and consequent difficulties in determining reactions involving such groups. Earlier, we introduced a procedure that depends upon the direct determination of the amount of hydrophobic substance absorbed or dissolved in the protein solution by a gas liquid chromatographic (GLC) technique. This procedure was employed to study the relationships of hydrocarbon structure and of protein structure in hydrophobic binding. Hydrocarbons used included alkanes, aromatic compounds, alicyclic compounds and methyl esters of fatty acids. In the present investigation, we have modified this procedure to permit the determination of interactions between triglycerides and proteins. The triglyceride is emulsified ultrasonically in water and equilibrated with a protein solution. The protein adsorbs to the surface of globules as a stabilizing interfacial layer. Relative stability of the emulsion is determined under standard conditions of filtration through a Nuclepore membrane. Either GLC or radioactive techniques are used to determine the amount of triglyceride stabilized by protein bound at the surface of the globules by hydrophobic bonds. Versatility of the procedure is demonstrated by comparisons of interaction of different proteins with trioctanoin and interaction of different triglycerides with bovine serum albumin. The order of binding of trioctanoin for a series of proteins (on a weight basis) as compared to bovine serum albumin taken as 100% was: casein, 162, bovine β-lactoglobulin, 132; chicken ovotransferrin, 118; chicken ovomucoid, 108; bovine γ-globulin, 96; β-amylase, 18; chicken ovalbumin, 3; α-chymotrypsin, 3; chymotrypsinogen A, 2; ribonuclease A, 2; and lysozyme, 1. It is concluded that the interaction of proteins with triglycerides is strongly dependent on protein structure.

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CHLORINE-LIPID REACTIONS OCCURRING DURING THE CHLORINATION OF FLOUR. H.B.S. Conacher, B.D. Page, and R.K. Chadha, Food Research Division, Health Protection Branch, New Research Centre, Health & Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada, K1A OL2.

Batches of freshly milled flour were chlorinated in the laboratory to provide several levels of chlorination up to and including those (0.1–0.2%) used commercially. Chlorine levels, measured in the flour by x-ray fluorescence spectroscopy (XRFS), before and after soxhlet extraction with diethyl ether, indicated approximately 40% of the added chlorine to be associated with the extracted lipids. Conversion of these lipids to methyl esters and examination of the latter by XRFS and by gas liquid chromatography indicated that chlorination of flour up to 0.2% resulted in (i) approximately 50% reduction of original oleate and linoleate, (ii) linear incorporation of chlorine into fatty acids up to 13%, and (iii) formation of varying amounts of dichlorooctadecanoates, dichlorooctadecenoates and tetrachlorooctadecanoates. The level of chlorine, determined by XRFS, in the flour, before and after extraction, and/or the methyl esters could be used to determine the level of chlorination of the flour.

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THE EFFECT OF BACTERIAL, VIRAL AND FUNGOUS INFECTION ON THE FATTY ACIDS AND AMINO ACIDS OF INSECTS. A.C. Thompson and P.P. Sikorowski, Boll Weevil Research Laboratory, P. O. Box 5367, Mississippi State, MS 39762.

Adult boll weevils, *Anthonomus grandis,* contaminated with *Streptococcus* sp., *Micrococcus varians,* and *Enterobacter aerogenes* (5000+ bacteria/weevil) showed a decrease up to 70% of fatty acids when compared to healthy weevils. Caloric evaluation of the fatty acids in the boll weevil egg indicates that the female weevil must provide 25% of her total body fatty acids per day in producing eggs (seven eggs/day). The total loss of amino acids was 18% in females and 22% in male weevils. Infection of *Heliothis virescens* in the larval stage with cytoplasmic polyhedrosis virus (CPV) resulted in a high larval mortality. In the early stages of CPV-infection there was a greater accumulation of lipids and fatty acids than in healthy larvae. In the pupae stage, the fatty acid requirement was limited: healthy pupae did not use any fatty acids in emergence to adults, whereas infected pupae did. Infected adult moths showed a decrease of fatty acids from 8% to 50% compared to healthy insects. The fatty acids used by the infected insects in emergence are attributed to the energy requirement of the virus. *Heliothis zea* infected with *Nosema heliothidis* showed a significant variation in lipids and fatty acids in the larval stage compared to the healthy larvae. In the pupal stage the change in fatty acids was not significantly different in the infected and healthy male pupae. However, in the infected female pupae the fatty acids declined rapidly with age. Infection of the bollworm, *H. zea,* with blue-green iridescent virus reduced the insects' ability to accumulate lipids by as much as 53% when compared to the healthy insect of the same age. Glycogen was reduced in the virus infected larvae as much as 70%, and the larval stage extended from 15 days to 30 days and the insect deprived of sufficient energy to emerge into pupae.

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PHASE BEHAVIOR OF ETHER LIPIDS FROM *CLOSTRIDIUM BUTYRICUM.* Howard Goldfine and Norah C. Johnston, 209 Johnson Pavilion/G2, 36th and Hamilton Walk, University of Penna. Philadelphia, PA 19104; Michael C. Phillips, Medical College of Pennsylvania.

The phospholipid class composition and alk-1-enyl acyl phosphoglyceride (plasmalogen) content of an anaerobic bacterium, *C. butyricum,* have been shown to undergo compositional changes when these cells are grown at varying temperatures, or at constant temperature with exogenous fatty acids under conditions that prevent endogenous fatty acid synthesis. These changes result in alterations in the thermal behavior of bilayers produced from the extracted lipids (Goldfine et al., 1977, Biochim. Biophys. Acta 488:341). To understand better how these changes may participate in the control of membrane fluidity, we have studied the plasmalogen form of phosphatidylethanolamine (PE), and a glycerol acetal derivative of the plasmalogen, which represents 26% of the total phospholipids in elaidate-grown cells and 49% in oleate-grown cells. These lipids were isolated from cells grown on elaidate in the absence of biotin. The acyl chains were 90% 18:1 and the alk-1-enyl chains were 99% 18:1, presumably the *trans* isomer. In liposomes formed from the pure plasmalogen, thermotropic phase transitions were observed at 30 C with the fluorescent probes *cis-* and *trans-*parinaric acids, and at 28 C by differential scanning calorimetry (DSC). This is about 6 C lower than the reported melting point of (diacyl) dielaidoyl PE. The elaidate-enriched glycerol acetal also underwent a broad melting transition at 31 C upon heating, but on cooling the transition to the gel state only commenced at about 17 C. This large hysteresis was observed with both parinaric acid probes and by DSC. The heating and cooling thermal transitions were partially resolved into two processes by DSC, and the sum of the enthalpy changes was considerably higher than that of PE. When increasing amounts of the PE fraction from elaidate-grown cells (30% diacyl and 70% alk-1-enyl acyl) were mixed with the glycerol acetal, the hysteresis was progressively reduced. These data suggest models for the control of membrane fluidity by altering the relative contents of plasmalogens, glycerol acetals, and diacyl lipids in the membrane of *C. butyricum.* (Supported by National Institutes of Health research grants AI-08903 and HL-22633)

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AUTOXIDATION OF METHYL ESTERS OF CYCLOPENTENYL FATTY ACIDS. E.M. Abdel Moety, Bundesanstalt fur Fettforschung, Piusalle 68. D-4400, Munster, (Westf) West Germany, and W.O. Lundberg,* P.O. Box 14422, Minneapolis, MN 55414 U.S.A.

The early stages of the autoxidation of methyl hyndnocarpate, chaulmoograte and gorlate in air have been examined at 40, 60, and 80 C, and the initial products have been compared by several methods with those derived from methyl oleate and linoleate autoxidized at 80 C. To supplement information about oxygen absorption and peroxide development in relation to time, other information about the early products, and some information about the reduced products, have been obtained by ultraviolet and infrared spectrometry, and thin layer chromatography. Some information about the structure of the initial peroxides has also been obtained by gas liquid chromatography of reduced peroxides in conjunction with mass spectral studies. The kinetic and other data presented herewith strongly support the conclusion that the cyclopentenylates yield initial autoxidation products that, although they are primarily peroxides, differ in some ways as was to be expected in the kinetics of their formation and their chemical nature from those of oleate and linoleate. Nevertheless, all of the data obtained strongly support the surmise that the peroxides are formed autocatalyicaly by chain mechanism, and that secondary products not derived from peroxide decomposition are formed *pari passu,* in lesser but increasing amounts with increasing temperature, probably from free radical intermediates. The autoxidation of cyclopententyl esters has potential importance in several ways, two of which are considered briefly.

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INVESTIGATION INTO THE PRO-OXIDANT NATURE OF CERTAIN TRANSITION METALS. P.E. Mountjoy, Liverpool Central Oil Co., Ltd., Oriel Street, Vauxhall Road, Liverpool L69 3AZ, England.

A discussion of the theory of metal catalyzed oxidation with allied rate dependence on level of contamination is proposed. The level of metal contamination is isolated into those proportions acting as a catalyst and those in a deactivated state. The use of chelating agents and their effectiveness is shown in context of specification levels for metals in an oil.

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RADICAL PROCESSES IN LIPIDS–A PULSE RADIOLYSIS STUDY. L.K. Patterson and M.S. Araos, Radiation Laboratory: University of Notre Dame, Notre Dame, IN 46556.

Radical induced lipid peroxidation is known to be dependent on the unsaturate nature of the lipid and on the degree of close-packing among molecules involved. In this study, fatty acid micelles have been used as simple model systems in which to examine the parameters governing oxidative degradation of polyunsaturated lipids. Individual steps in the radical mechanism have been time resolved for a variety of systems by pulse radiolytic techniques and are shown to vary markedly between micelles and homogeneous solutions of surfactant monomer. Initiation, propagation and termination may also be seen to vary with alteration in the unsaturated structure of these acids. Such techniques have been further applied to determine reaction rates between peroxy radicals and lipid directed antioxidants such as α-tocopherol.

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SEPARATION OF LIGHT FRACTIONS OF SOYBEAN AND SOYBEAN MEAL IN BULK STORAGES AND INFLUENCE OF IT ON THE QUALITY OF SOYBEAN OIL, LECITHIN AND SOYBEAN MEAL. Dusko Jericevic, Tvornica za preadu soje p.o. (TPS), 57000 Zadar-Gazenice pp215, Yugoslavia.

Experience shows that a soybean crushing plant with modern technology could still have a great problem with quality variations of soybean products caused by the handling of soybeans and soybean meal in bulk storages even if the plant processes soybeans of good and uniform quality. This problem is discussed in two parts, substantiated with laboratory analytical data. The first part deals with soybeans in bulk storage where, due to a specific way of loading, the separation of light fractions (dust, hulls, parts of broken beans, shrunken dry beans, foreign material-like leaves, etc.) occurs. These fractions accumulate in the storage forming a belt around the pile of soybeans which, in certain moments, enters the processing line causing difficult separation (cleaning) and poor extraction in that miscella and distilled oil filter very poorly with serious filter plugging; oil contains impurities (mechanical impurities, proteins and carbohydrate extraction residues, etc.) that influence the color changes of the oil; oil degumming is difficult and lecithin produced is of low grade quality due to the high content of impurities (benzene insolubles reach 4%); and storage stability of degummed oil and lecithin is reduced. The second part of the paper discusses almost identical problem which occurs with soybean meal in bulk storage. The meal leaving the production and entering storage from above, distributes unevenly causing the quality of light fraction of meal to be very well under the standard regulations (e.g., 32% proteins). The quality of the heavier fraction usually satisfies the quality regulations as far as protein content is concerned, but with high oil content, due to poor extraction, contributes to already existing potential hazards caused by enormous dust accumulation in bulk storages.

380A
THE EFFECT OF PHOSPHOLIPID ON BINDING PROPERTIES OF A RECONSTITUTED INSULIN RECEPTOR. R.J. Gould and A.A. Spector.

The insulin receptor from turkey erythrocytes has been reconstituted after solubilization with 1% β-octylglucoside, a nonionic detergent. The detergent was removed in the presence of soybean phosphatidylcholine and bovine brain phosphatidylserine. After reconstitution, 50% of the fatty acyl portions of the phospholipid associated with the insulin binding activity contained two or more double bonds. This compared to only 30% in the native membranes. The properties of the reconstituted receptor were studied at 15 C in a buffer containing 85 mM Tris-HCl, 30 mM NaCl, 10 mM glucose, 1 mM EDTA, pH 7.8. The reconstituted receptor was saturable and bound insulin specifically. The binding was dependent on the time of incubation and the amount of protein in each incubation. Proinsulin and DOP-insulin were competitive with porcine insulin at concentrations relative to the published bioeffects, (porcine insulin>proinsulin>DOP-insulin). When binding data was analyzed by the method of Scatchard, a curvilinear plot was obtained. The $\bar{K}_e$ obtained from this plot was 3.3×10^8 M^{-1}. This was very close to the $\bar{K}_e$ of 2.8×10^8 M^{-1} for native membranes. The reconstituted system had a 7.5 fold greater $\bar{K}_f$ than the native system (9.2×10^7 M^{-1} vs. 1.2×10^7 M^{-1}). The dissociation of tracer amounts of insulin from the reconstituted receptor was studied in the presence and absence of 1 μg/ml unlabeled insulin. No difference was seen between these two conditions. This is in sharp contrast to the native system where tracer amounts of labeled insulin dissociate more readily in the presence of an excess of insulin than in the absence. Experiments are currently in progress to determine whether the degree of unsaturation of the surrounding lipid plays a role in this dissociation phenomenon.

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PREFERENTIAL PHOSPHOLIPASE A_2 ACTIVITY AGAINST OXIDIZED PHOSPHATIDYCHOLINE. Alex Sevanian, Robert A. Stein, and James F. Mead, Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, Los Angeles, CA 90024.

There has been considerable speculation concerning the fate of damaged lipids in biomembranes. It has been suggested that the processes leading to membrane damage and leakage may involve either loss of integrity via lipid peroxidation, or that such events may accelerate enzyme mediated lipolysis resulting in membrane destruction. The possibility that oxidized fatty acids in membranes may be recognized and preferentially removed by lipolytic enzymes such as phospholipase A_2 (PL A_2) has been difficult to demonstrate directly. Part of this difficulty resides in the instability of lipid peroxidation products and hence in the unavailability of suitable substrates. There is now evidence that lipid peroxidation in membranes may yield products unlike those found in other lipid systems; in many instances lipid epoxides represent the major isolable products of peroxidation. The isolation and characterization of phospholipid epoxides from peroxidized liposomes, microsomes and whole organs, and their relative stability in aqueous preparations prompted an analysis of PL A_2 activity using phospholipid epoxides as substrates representing peroxidation products. Using 1-palmitoyl-2-epoxystearoyl phosphatidylcholine (PCE) prepared from 1-palmitoyl-2-oleoyl phosphatidylcholine (PC), we were able to demonstrate that PL A_2 was at least twice as active against PCE than PC in either pure enzyme (snake venom) or microsomal preparations. The major products of the PC/PCE-PL A_2 reaction were lyso PC, free fatty acid or fatty acid epoxide. The fatty acid epoxide, but not PCE, was rapidly hydrated in the absence of an epoxide hydratase inhibitor. The results of these studies will be discussed.

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MODIFICATION OF POLYUNSATURATED FATTY ACIDS DURING PARTIAL HYDROGENATION OF MENHADEN OIL WITH NICKEL CATALYST. J-L. Sebedio, R.G. Ackman, Nova Scotia Technical College, P.O. Box 1000, Halifax, Nova Scotia B3J 2X4, Canada.

Ten samples were collected during a pilot scale hydrogenation of menhaden oil with nickel catalyst (0.2%) as the iodine value was being reduced from 159 to 84.5. The evolution of the total *trans* content as a function of the degree of hydrogenation was followed by infrared spectroscopy. The total *trans* content, calculated as methyl elaidate, increased from 3.4% at IV 150 to 24.9% at IV 120.5 and to 45.1% at IV 84.5. Comparisons of gas liquid chromatographic analyses of the hydrogenated samples on wall-coated open tubular columns (Silar-5CP or Apiezon-L) showed that no precise quantitative analysis could be obtained directly from the chromatograms due to the considerable overlap of the isomeric artifact polyunsaturated fatty acids. The formation of bromomercuric adducts of the total fatty acid methyl esters, followed by thin layer chromatography, permitted the separation of these complex mixtures into monoene, diene, triene, tetraene and pentaene fractions. The total fatty acid composition was reconstructed by using heptadecanoic acid (17:0) as an internal standard. The effects of the hydrogenation of menhaden oil, terminated at IV 84.5, are: a large decrease in tetraenes (5.6% at IV 159, 0.4% at IV 84.5), pentaenes and hexaenes (8.8% at IV 159, trace at IV 84.5); a large increase in monoenes (23.9% at IV 159, 34.2% at IV 84.5), dienes (3.9% at IV 159, 13.2% at IV 84.5% and trienes (4.2% at IV 159, 8.3% at IV 84.5). Only a slight increase in saturates (41.6% at IV 159, 43.8% at IV 84.5) was observed. The increase in monoenes is especially important for eicosenoic (20.1) and docosenoic (22:1) isomers (1.3% to 4.5% for 10:1 and 0.2% to 1.7% for 22:1). The GLC analyses on Apiezon-L indicate that a major proportion of the newly formed monoenes have a *trans* configuration. The difference in oxidative stability of the hydrogenated samples, assessed by peroxide value determination, is evident after two months of storage. The peroxide value for IV 150.0 increased from 4.2 to 7.6 and for IV 84.5 from 0.4 to 2.0.

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ANALYSIS OF "ESSENTIAL" PHOSPHOLIPIDS (EPL), A CLINICALLY USED PHARMACEUTICAL. Joseph G. Turcotte, John Y.-K. Hsieh, and David K. Welch, Department of Medicinal Chemistry, College of Pharmacy, University of Rhode Island, Kingston,

RD 02881.

"Essential" phospholipids (EPL) is a pharmaceutical claimed to be effective for the therapy of a number of clinical disorders; studies exist on the evaluation of EPL therapy in patients with hyperlipidemias and hyperproteinlipidemias, ischaemic heart disease, blood pressure and arteriosclerotic circulatory disturbances, diabetic vascular disease, and renal insufficiency. Phosphatidylcholine was identified as the major component (ca 60%) of EPL using thin-layer and high pressure liquid chromatography. Neutral lipids, phosphatidylethanolamine, lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidylinositol, 7-(β-hydroxyethyl) theophylline, and methyl linoleate (tlc/glc) also were identified. The phosphatidylcholine ("purified EPL") component of EPL was isolated and purified by column and thin-layer chromatography. Its molecular and stereochemical properties were studied by chromatography, chemical means, high resolution PMR spectroscopy, and optical rotation. The total fatty acid compositions of "purified EPL" and purified ordinary soy lecithin ("purified SL") were determined and compared; oleic and linoleic acids constituted approximately 70% of the total unsaturated fatty acids in both "purified EPL" and "purified SL". Determination of the positional distributions of fatty acids of "purified EPL" showed that saturated (16:0, 18:0), oleic, and linoleic acids were esterified mainly at the *sn*-1 positions and unsaturated fatty acids at the *sn*-2 positions of the molecules; linoleic acid was the predominant esterified fatty acid at both the *sn*-1 (53%) and *sn*-2 (83%) positions of "purified EPL." High pressure liquid chromatography (reverse phase) of phosphatidic acid dimethyl esters (PADE) derived from "purified EPL" via cleavage with phospholipase D and reaction of the corresponding phosphatidic acids with diazomethane, showed dilinoleoyl phosphatidylcholine to be the most abundant individual molecular species of "purified FPL," constituting approximately 50% of the phosphatidylcholine molecules; fatty acid pairing of molecular species of "purified EPL" also was estimated by gas liquid chromatography of derived diacylglycerol acetates. The results of the analyses has provided a basis for discussion of certain factors that should be considered in therapy for human use with classes of natural-product phosphoglyceride drugs such as "essential" phospholipids.

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IMPROVED PROCEDURE FOR TITRATING CYCLOPROPENE ESTERS WITH HYDROGEN BROMIDE. R.O. Feuge, Louis P. Codifer and J. Hampden Zeringue Jr., Southern Regional Research Center, USDA, SEA, P.O. Box 19687, New Orleans, LA 70179.

Previously proposed methods for determining cyclopropenes in cottonseed oil and other vegetable oils by titration with hydrogen bromide are not entirely satisfactory. The acetic acid sometimes used as a solvent reacts with cyclopropenes in the presence of hydrogen bromide. When non-polar solvents are used, the acid-base indicators suggested heretofore form insoluble complexes. Impurities, including mono- and diglycerides and peroxides, normally present in vegetable oils, interfere with the titration. Purification by treatment with large proportions of alumina, used heretofore, disproportionates the esters being analyzed. Relatively simple procedures have been devised for interesterifying a deteriorated or crude oil to yield methyl esters of high purity. An evaluation of possible indicators for the titration established that 4-phenylazodiphenylamine had the best characteristics, including great sensitivity at 25 C to hydrogen bromide. The best procedure was to titrate purified methyl esters or triglycerides with hydrogen bromide at 60–65 C to just past the end point, cooling to about 25 C, and back titrating with aniline in toluene. At a cyclopropene level of 1%, reproducibility was within ± 0.003%.

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A TWO-STAGE, ONE-DIMENSIONAL THIN LAYER CHROMATOGRAPHIC METHOD FOR SEPARATION OF LIPID CLASSES. Joel Bitman, D.L. Wood and J.M. Ruth, U.S. Department of Agriculture, Bldg. 309, Beltsville Agricultural Research Center, Beltsville, MD 20705.

A thin layer chromatographic (TLC) technique was developed for the routine analysis of lipid classes of blood, milk, tissue and egg yolk. This procedure provided rapid and reproducible separations suitable for in situ quantitation by densitometry. Emphasis was placed on using standard, commercially available equipment. Non-uniform distribution of solute was observed in some instances of sample application. A schematic model and mathematical treatment of this uneven solute distribution are described. The spotted TLC plate was first developed in chloroform methanol acetic acid (98:2:1) to 16 cm. After air drying, the plate was developed in hexane ethyl ether acetic acid (94:6:0.2) to the top of the plate. After air drying, the plate was dipped into a solution of 3% cupric acetate in 8% phosphoric acid for 3 seconds, and heated at 130 C for 30 minutes to char the separated lipid classes. The amount of lipid in the charred spots was quantified by densitometry in situ using a TLC scanner at 400 nm. The method was applied to lipids from: a) cow, calf, rabbit, chicken, lamb and rat plasma; b) milk, corn oil, egg yolk; and c) chicken, cow and rat liver.

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FATTY ACID DATA, THEIR VARIABILITY WITH ANALYTICAL METHODOLOGY AND THEIR COLLATION BY COMPUTER. John L. Weihrauch, USDA Consumer and Food Economics Institute, 6505 Belcrest Road, Hyattsville, MD 20782.

Information on the amounts of 19 fatty acids in foods is now included in the revised and expanded national tables of food composition which are published in sections, by food group, in Agriculture Handbook No. 8, "Composition of Foods... Raw, Processed, Prepared." The number of fatty acids and their relative distribution reported in a food may vary considerably among laboratories, depending on analytical methodology. Analytical factors affecting the fatty acid composition are reviewed, and the problems in collating data from various sources are discussed. Described is a computer-based procedure for summarizing data on fatty acids in terms of the weight percent of their methyl esters and for converting the relative values to amounts of each fatty acids (as the free acid) in 100 grams of fat and/or 100 grams of food.

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CHROMATOGRAPHIC SEPARATION OF THE STEREOISOMERS OF ALPHA-TOCOPHEROL. Hal T. Slover and Raymond H. Thompson, Jr., Nutrition Institute, Nutrient Composition Laboratory, Building 264, Room 27, BARC-East, Beltsville, MD 20705.

Totally synthetic alpha-tocopherol, *all-rac*-α-tocopherol (commonly referred to as dl-α-tocopherol) made from synthetic phytol or isophytol, is a mixture of eight stereoisomers consisting of four racemates in unknown proportions. This is the synthetic form of vitamin E added to foods or otherwise used as a vitamin supplement. The semisynthetic alpha-tocopherol made from natural phytol, 2-ambo-α-tocopherol (also commonly known as dl-α-tocpherol), is a mixture of two diastereoisomers, thought to closely approach equimolar proportions. The acetate of 2-ambo-α-tocopherol is the international standard for vitamin E activity. No analytical separation of the stereoisomers of these tocopherols has previously been reported. The diastereoisomers of 2-ambo-α-tocopherol have been completely separated as TMS ethers by gas chromatography on a 100m × 0.25mm glass capillary column coated with SP2340. In the same way, *all-rac*-α-tocopherol has been partially separated into four peaks, which coincide with the four diastereoisomers of 4′-ambo-8′-ambo-α-tocopherol, produced by the hydrogenation of natural alpha-tocotrienol. Retention data and peak areas for the diastereoisomers of synthesized alpha-tocopherols and commercial products have been determined; limited data on the isomers of other tocopherols will also be presented.

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DETERMINATION OF LIPID CLASSES, USING SUBMICROLITER AMOUNTS OF PLASMA, BY THIN-LAYER CHROMATOGRAPHY AND "IN SITU" SPECTROFLUORIMETRY AFTER THERMAL TREATMENT IN THE PRESENCE OF SILICON TETRACHLORIDE. Ramon Segura and Xavier Navarro, Departamento de Fisiologia, Facultad de Medicina, Universidad Autonoma de Barcelona, Bellaterra (Barcelona) Spain.

In a previous paper (Segura and Gotto, J. Chromatog. *99*(1974) 643), we have described a procedure for the detection and quantitation of organic compounds on thin-layer chromatoplates after inducing their transformation into fluorescent derivatives by thermal treatment of the chromatoplates in the presence of ammonium hydrogen carbonate. The method has been applied to the determination of lipids using microliter quantities of plasma ((Segura and Gotto, Clin. Chem. *21*(1975)991) (Kupke and Zeugner, J.Chromatog. *146*(1978)261)). This procedure required several hours of heating at 150 C and, in most instances, precluded the use of commercial plates. After a systematic investigation of many different types of ammonium derivatives and of several protonating reagents, we have found that heating the chromatoplates in the presence of silicon tetrachloride induces the formation of fluorescent derivatives of all classes of lipid compounds in less than an hour, using either commercial or hand-made plates of silica gel and other types of inorganic adsorbents. This new procedure shows greater sensitivity than that obtained by the ammonium hydrogen carbonate method, allowing the determination of all lipid classes in organic extracts corresponding to 0.1–0.2 μl of plasma. Regardless of the chemical peculiarities of the original compound, all the fluorophores exhibit the same excitation maximum (385 nm) and the same emission maximum (460 nm); the fluorophores are stable for months. In all cases, there appears to be a linear relationship between the square root of the peak area and the amount of compound over a fairly broad range of concentrations. The lower limits of detection range from 10 ng for cholesterol to 50 ng for triglycerides. This procedure is not specific for lipids for all kinds of organic compounds are transformed into fluorescent derivatives after heating in the presence of silicon tetrachloride.

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A GAS CHROMATOGRAPHIC METHOD FOR QUANTITATIVE DETERMINATION OF LONG CHAIN NON-ESTERIFIED FATTY ACIDS IN HUMAN SERUM. Michael Hockel, Wolfgang Dunges, Anne Holzer, Peter Brockerhoff and Gunter H. Rathgen, Universitatsfrauenklinik, Langenbeckstrasse 1, D-6500 Mainz, West Germany.

Non-esterified fatty acids (NEFA) from C_{12} to C_{24} are assayed in human serum in a four step procedure: extraction, volume reduction, methylation and gas chromatography. NEFA are extracted from 100 µl of buffered serum with chloroform heptane methanol. After adding ethyl acetate the volume of the extract is reduced under partial reflux to 5 µl, the NEFA being concentrated twenty-fold in relation to their serum concentrations. Thus compounds as lauric acid, linolenic acid or arachidonic acid which are present in serum only in minor amounts can subsequently be quantitatively determined too. After the addition of methyl iodide, potassium carbonate and a crown ether to the dry ethyl acetate solution the methyl esters are prepared with a yield of 100% by heating in a microrefluxer for 5 minutes. Gas chromatography is carried out with 1 µl of the reaction mixture on a 6 ft × 1/4 in. glass column packed with 10% SILAR 10 CP on Chromosorb W-HP by temperature-programmed operation. The coefficients of variation for 20 determinations of a serum were 2.7% for the total NEFA content and 3% to 10% for individual NEFA depending on their relative amount.

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DETECTION OF HYDROXY FATTY ACIDS IN BIOLOGICAL SAMPLES USING CAPILLARY GAS CHROMATOGRAPHY IN COMBINATION WITH POSITIVE AND NEGATIVE MASS SPECTROMETRY. H.-J. Stan, Institut f. Lebensmittelchemie, Müller-Breslau-Str. 10, 1000 Berlin 12, Germany; M. Scheutwinkel-Reich, Tech, Universität, Berlin.

Hydroxy fatty acids are formed from fatty acids via enzymatic reactions, through autoxidation and during heating of fats in the presence of air (deep frying). Furthermore, hydroxy fatty acids are components of various cell membranes and they can be utilized to elucidate the structure of unsaturated fatty acids by specific oxidation to locate the position of the double bonds. The most common method for use in the structural analysis of these substances is the GC/MS analysis of trimethylsilyl ethers of the methyl esters using electron impact ionization. Comparing electron impact with chemical ionization mass spectrometry, the latter is the superior technique. All ions necessary for structural analysis are observed at sufficiently high levels of intensity when methane or isobutane are used as reactant gases. The molecular weight can be determined from the ion group M+H, M-15, and M+H-90. The ionic series M+H-n×90 enables one to determine the number of hydroxy groups. The position of the hydroxy groups can be derived from the fragments of the α-cleavage of the fatty acid chain. The application of heptafluorobutyrates as derivatives for hydroxy fatty acid methyl esters shows advantages in the trace analysis of these compounds. Using a simple electron capture detector the determination of hydroxy fatty acids in biological samples is possible at pg levels. Heptafluorobutyrates exhibit useful mass fragmentation patterns in the positive as well as in the negative chemical ionization mode. With methane as the reactant gas M+H usually is base peak in positive mass spectra. The ionic series M+H-n×214 leads to the number of hydroxy groups in the molecule. In the negative mass spectra M and M-20 are indicative for the molecular weight. The ion group m/z 213, 194, and 178 at high levels of intensity is typical for heptafluorobutyrates. The advantage of the application of heptafluorobutyrates is the high sensitivity which can be obtained in trace analysis using electron capture detection or negative mass spectrometry.

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A NEW METHOD FOR THE DETERMINATION OF SMOKE POINT. A. Seher and F. Bregulla, Federal Center for Lipid Research, Piusallee 68–76, D-4400 Muenster, Germany.

Besides other methods, the determination of smoke point is used to control frying fats. It was found that the formation of smoke over the open Cleveland cup during heating of fat is caused by condensation of the volatiles when the saturation concentration in the air is exceeded. This concentration depends not only upon the composition of the volatiles, but also upon the temperature of the laboratory and obviously other atmospheric conditions. Moreover, the reading of the smoke point temperature depends on subjective impressions of the experimentator. These facts decrease the repeatability and the comparability of the results. To improve the determination, a closed smoke point apparatus was developed. The fat is heated in a glass vessel, from which an intermittent stream of nitrogene blows the volatiles from the head space into a temperature controlled box against a light beam. The occurrence of smoke is registered automatically by photocells and a recorder. The results obtained with the new apparatus are independent from individual influences as well as atmospheric ones. Therefore, the comparability of the determination is increased from a deviation of more than 20 C, obtained formerly, to a value less than 4 C.

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RELATIONSHIP BETWEEN RAPESEED CHLOROPHYLL, RAPESEED OIL CHLOROPHYLL AND PERCENTAGE OF GREEN SEEDS IN RAPESEED. James K. Daun, Canadian Grain Commission, Grain Research Laboratory, Room 1308-303 Main St., Winnipeg, Manitoba, R3C 3G9.

Greenness is the major degrading factor in Canadian rapeseed and rapeseed oil. Samples of rapeseed and rapeseed oil collected from Western Canadian crushing plants showed that about 65% of the seed chlorophyll showed up in the finished oil. The chlorophyll contents in the oils varied from 3 to 30 ppm while the chlorophyll in the seed varied from 1.5 to 23 ppm. The percentage green seeds as used in the Canadian grading system correlated with the oil and seed chlorophyll ($r = 0.7$). Recommendations are made for seed chlorophyll, oil chlorophyll and percentage green seeds to correspond with rapeseed oil color standards.

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DESIRED QUALITY ATTRIBUTES IN WINTER AND SUMMER RAPESEED. Alfred Thomas, Unilever Ltd., F. Thörl's, Vereinigte Harburger Oelfabriken, 1. Hafenstr. 15, 21 Hamburg 90, Federal Republic of Germany.

Quality assurance programs have played a significant role in rehabilitating and in maintaining the importance of rapeseed and its products. In Europe the processing of indigenous and Canadian rapeseed ensures uniform plant utilization throughout the year, which in itself contributes to improving quality. Indigenous rapeseed is predominantly of the high-yielding winter type. The European oil mills have learned to adapt to differences in low erucic acid seed characteristics during processing. Especially the influence of different conditioning and pre-expelling parameters on the characteristics of expeller and extraction oil is continuing to receive attention. Sulfur compounds, phosphatide content, color, and analytical oxidation values are important criteria for the required refining techniques, which can range from classical methods to physical refining, and the quality of the fully refined product. Whereas low erucic acid rapeseed oil can generally by regarded as an alternative to soybean oil, crystallization behavior of hardened products can differ significantly, this appearing to be a function of fatty acid composition and sulfur compounds. Progress is also being made in upgrading meal quality, especially for application in poultry feed, by developing cultivars with glucosinolate levels below 1 o/o. Such new varieties might also contribute to reducing the problem of fishy egg taint observed with some breeds of layers. Decreased rumen degradability of rapeseed meal by treatment with formaldehyde may further improve flexibility of use. Various established and potential quality attributes for rapeseed, meal, and oil will be discussed and summarized.

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RAPESEED OIL PROCESSING IN CANADA. T.K. Mag, Canada Packers Ltd., 2211 St. Clair Ave., W., Toronto, Canada M6N 1K4.

The growing of rapeseed in Canada has undergone significant changes in the last decade. Important improvements, made to the crop by plant-breeders, have changed the character of oil and meal. The improvements have led to the choice of the name "canola" to distinguish the new oil (and meal) from that of the traditional seed. Canola oil is now the most important crude oil processed for edible purposes. The processing steps used are the same as those practiced on soybean oil, for example, or most other oils. Certain processing details, however, differ from those used for other oils to allow for differences in impurities found in the oil, and differences in fatty acid composition. Current processing practice and how it differs from that applied to other oils will be described. Also, the possibilities for applying refining to improve processing economics and reduce water pollution problems will be discussed.

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EFFECT OF RAPESEED OIL USED AS A DUST INHIBITOR ON THE COMPOSITION OF WHEAT LIPIDS. James K. Daun and Fu-hung Hsieh, Canadian Grain Commission, Grain Research Laboratory, Room 1308-303 Main St., Winnipeg, Manitoba, Canada, R3C 3G9.

Rapeseed oil was shown to effectively lower the airborne dust concentration when sprayed on wheat in concentrations of as little as 0.05% (w/w). Much of the dust was found adhering to the treated kernels. On milling, the oil was found primarily in the bran and shorts fractions. Rapeseed oil and dust caused an increase in flour color and a slight increase in ash content. Changes in color were in proportion to the amount of oil present. The fatty acid composition of the wheat lipids was changed by addition of rapeseed oil.

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MICROWAVING RAPESEED TO INACTIVATE MYROSINASE AND ITS EFFECT ON OIL AND MEAL QUALITY. P.N. Maheshwari, D.W. Stanley and F.R. van de Voort, Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

Rapeseed was dehulled using a Palyi pneumatic attrition system which produced 62–66% clean dehulled meat. Dehulled rapeseed was preconditioned to 7, 10 and 13% moisture levels, exposed to microwave irradiation for periods of up to 2.5 min. and analyzed for residual thioglucoside glucohydrolase (myrosinase) activity. The 7% moisture samples heated slowly and required at least a 2.5 min. treatment while 10 and 13% moisture samples heated more rapidly and required microwave exposures of 1.5 min. or less for complete inactivation of the ehzyme. The sulfur content of oils obtained from adequately microwave-treated samples was equal or lower than commercially processed crude rapeseed oils. The shorter microwave treatment of dehulled rapeseed produced considerably lighter oils and did not adversely affect the color of the mean. It also destroyed some of the rapeseed glucosinolates and improved the meal palatability. However, goitrogenic properties of microwave-treated rapeseed meal evaluated by mice feeding experiments did not appear different from untreated rapeseed meal.

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PROCESSING FACTORS AFFECTED BY SULFUR-CONTAINING COMPOUNDS OF RAPESEED. Antoni Rutkowski, Stanisław Gwiazda and Krzysztof Krygier, University of Warsaw (SGGW), ul. Grochowska 272, 03-849 Warszawa, Poland.

A characteristic feature of cruciferous oilseeds is a high level of sulfuric compounds as sulfur-containing aminoacids and thioglycosides which determine the nutritional value of rapeseed meal and affect processing factors in oil mills. During rapeseed processing, products of thioglycoside splitting are liberated and attack metal and, as a result, a specific sulfur corrosion of oil mill equipment develops. The most exposed are cooker, toaster and transporters of wet meal. The results of industrial investigation of sulfur corrosion in rapeseed oil mill laboratory investigations of this phenomenon are described and the sensitivity to corrosion of several construction steels during rapeseed processing is considered. The negative effect of a high content of sulfur-containing compounds in starting oil significantly depressed the nickel catalyst activity and influenced the kinetics of rapeseed oil hydrogenation. Removing of these compounds by refining and its influence on the rate of oil hydrogenation was discussed.

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PHENOLIC ACIDS IN RAPESEEDS. H. Kozlowska, D. Rotkiewicz, R. Zadernowski, Institute of Food Engineering and Biotechnology, University of Agriculture and Technology, Olsztyn, Poland; and F. Sosulski, University of Saskatchewan.

Phenolic acids in different rapeseed varieties: Skrzeszowicki (high erucic and glucosinolates), Janpol (low erucic, high glucosinolates), Start and Tower (zero erucic, low glucosinolates), Candel (zero erucic, low glucosinolates with yellow hulls), were determinated. Total amount of phenolic acids in investigated seeds was between 2.3–3.3% as dry weight of meal. Identified in all seeds examined, the following phenolic acids were identified: salicylic, p-hydroxybenzoic, vanillic, gentisic, o-coumaric, syringic, p-coumaric, sinapic and ferulic. Among the identified phenolic acids, sinapic acid dominated although 80% of it was bound with choline.

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AUTOXIDATION PROCESSES IN THE SYSTEM RAPESEED OIL/LECITHINE. Henryk Niewiadomski, Politechnika Gdańsk ul. Majakowskiego 11/12, 80-952 Gdańsk (Poland).

Under the meaning of the quality of rapeseed oil and rapeseed lecithin we include the organoleptic properties which determine the range of the utilization of those products in the food industry. Their quality is affected by genetic reasons, errors during the production, and by autoxidation. Leaving aside the two first groups of reasons, this paper presents the results of studies on the effect of rapeseed oil autoxidation on the formation of melanophosphatide colors and the effect of phospholipids on the autoxidation of rapeseed oil. Melanophosphatides reduce the quality not only of lecithin color, but also its taste and odor. On the other hand, two main fractions obtained from commercial "lecithin" that is lecithin and cephaline fractions introduced to rapeseed oil, inhibit its autoxidation, the lecithin fraction being more active. Thus the system oil-rapeseed lecithin approaches the state of some equilibrium in the process of progressing deterioration of its organoleptic properties.

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FACTORS AFFECTING QUALITY OF CRUDE AND REFINED RAPESEED OIL. Christina Kristoffersson, AB Karlshamns Oljefabriker, 2-292 00, Karlshamn, Sweden, and Josef Dahlén, Swedish Oil Extraktion, Ltd.

The quality of crude and refined oil is to a large extent dependent on the quality and previous history of the seed. The processing conditions during extraction and refining also contribute to the final result. The paper will deal with some experiences from full-scale and laboratory tests. Among other things the variation in phosphatide and sulfur contents and the effect of these variations will be discussed.

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PHYSICAL REFINING OF RAPESEED OIL. James J. Ledden, James J. Ledden Ltd., 1550 de Maisonneuve Blvd. W., Suite 1165, Montreal, Que. H3G 1N2 Canada.

Rapeseed oil from selected stocks has been commercially refined by the physical refining method. A large number of samples of crude rapeseed oil from different geographic locations, various seed varieties and of different general quality has been laboratory-processed and plant operating parameters established. An acid pretreat to enhance degumming is done using a citric/phosphoric acid mixture and an acid-treated clay is used for bleaching. The laboratory and commercial plant scale results are described. This is an ongoing project and the direction for further work which would widen the usefulness of the method is indicated.

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FORMATION OF FLAVOR VOLATILES BY THE SELECTIVE OXIDATIVE CLEAVAGE OF UNSATURATED FATS. P.A.T. Swoboda and K.E. Peers, A.R.C. Food Research Institute, Colney Lane, Norwich, NR4 7UA, England.

This introductory paper for the section on flavor and flavor stability of fats and fat-containing foods will review some aspects of the chemical as well as the enzymic reactions involved. Not only is there selectivity in sensory terms, but there is evidence for selectivity in both the oxidation of unsaturated fats and the formation of flavor volatiles by cleavage of the first formed hydroperoxides. Results of the authors' research, as well as those from the scientific literature, will be discussed.

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FLAVOR AND OXIDATIVE STABILITY OF HYDROGENATED AND UNHYDROGENATED SOYBEAN OIL: EFFECT OF TERTIARY BUTYL HYDROQUINONE. T.L. Mounts, K. Warner, and G.R. List, Northern Regional Research Center, AR, SEA, USDA, 1815 N. University, Peoria, IL 61604.

The efficacy of tertiary butyl hydroquinone (TBHQ) treatment for enhancement of the storage stability of soybean oil has been studied by flavor evaluation and chemical analysis. Soybean oils (a) unhydrogenated (IV = 137.7; % linolenate = 8.3), (b) hydrogenated with nickel catalyst (IV = 109.1; % linolenate = 3.3), and (c) hydrogenated with copper-chromium catalyst (IV = 112.8, % linolenate = 0.4) were each deodorized. In the cooling stage of the deodorizer each oil was treated with citric acid plus TBHQ. These freshly deodorized oils were compared to separate batches of each oil treated with citric acid alone or with citric acid plus BHA/BHT. An analytical taste panel performed sensory evaluations by paired comparisons of samples. The oils were also evaluated after being subjected to accelerated storage tests (4 days and 8 days at 60 C) and a fluorescent light exposure test (4 hr, ambient temperature). Oxidative stability was measured by 8-hour Active Oxygen Method (AOM) procedures and peroxide value determinations at time of flavor evaluation. Oxidative stability, as measured by the AOM, was improved by hydrogenation and TBHQ. The flavor stability of the three oils was not enhanced with added TBHQ under any test conditions.

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CORRELATION OF GAS LIQUID CHROMATOGRAPHIC VOLATILES WITH FLAVOR INTENSITY SCORES OF STORED SUNFLOWERSEED OILS. W.H. Morrison, B.G. Lyon and J.A. Robertson, USDA, SEA, Russell Research Center, Field Crops Laboratory, P.O. Box 5677, Athens, GA 30604.

Samples of sunflowerseed salad oil from seed produced in the northern U.S. containing BHA, BHT, TBHQ, and propyl gallate were stored in flintglass and amber bottles in the presence and absence of light for 16 weeks. Using Dupuy's direct GLC method, correlations were made between pentane and total volatiles and flavor intensity scores as well as flavor intensity values (FIV's). Samples stored in clear bottles exposed to light had higher scores and pentane content than those stored in amber bottles or in the dark. Peroxide values were highest for samples stored in amber bottles. High correlation was found between flavor intensity scores and pentane and between pentane and FIV's for rancid and painty flavor discriptors. There was some indication from the chemical and sensory data that suggested differences between antioxidant efficacy.

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FAT OXIDATION IN DRY WHOLE MILK: SIMULTANEOUS DIFFERENT ANTIOXIDANTS. Gunnar Hall, SIK-The Swedish Food Institute, Fack, S-400 23, Gothenburg, Sweden.

Fat oxidation in whole milk powder containing different antioxidants was studied. The powder was stored at 25 C in either air or nitrogen. The formation of volatile oxidation products was studied by gas chromatography and mass spectrometry. Two different headspace sampling techniques were used. The most volatile compounds were analyzed by an equilibrium sampling method while the less volatile compounds were analyzed by a continuous sampling method whereby large headspace samples could be taken. Sensory changes in the milk powder due to fat oxidation analyzed by odor and flavor profiling techniques. Computer techniques have been used to treat the data statistically. In this paper the formation rates of different volatile oxidation products stored under different conditions will be discussed. Also the sensory changes and the relationship between sensory changes and formation of different oxidation products will be discussed.

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STORAGE OF BUTTER AND FAT-CONTAINING ANIMAL FOODS AT LOW TEMPERATURES. K. Porsdal Poulsen and F. Lindelöv, Technical University of Denmark, DK2800 Lyngby, Denmark.

Often it is assumed that any lowering of temperature will improve stability of the organoleptic properties of foods. A number of studies, however, show that when freezing and crystallization occur, stability is influenced by concentration of reactants dissolved in the unfrozen part of the water and/or fat phase. As the temperature is lowered, a smaller part of the two phases is in a liquid state, which results in an increase in reaction rate. This increase is counteracted by the temperature influence at the reaction rate constants, so the final result is difficult to predict. Our studies comprise temperatures between 5 C and –60 C and large differences are found, not only in stabilities expressed in day, but also in shape of the resulting temperature-stability curves. Organoleptic tests and chemical analysis are used to estimate stability of flavors.

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A COMPARISON OF VOLATILE FLAVOR COMPOUNDS IDENTIFIED IN EXPELLED AND PRESSED COCOA BUTTER. I. BASIC COMPOUNDS. Ken N. Lee, James T. Carlin, Oliver Hsieh and Stephen S. Chang, Department of Food Science, Rutgers State University, P.O. Box 231, New Brunswick, NJ 08903; Lucy S. Hwang, National Taiwan University.

The differences between the volatile flavor compounds (VFC) of the basic fraction of the expelled and pressed cocoa butters were studied. The VFC were isolated from cocoa butters by the use of a continuous countercurrent vacuum steam distillation apparatus specially deisgned in our laboratory. The VFC, when added back to a deodorized cocoa butter, yielded a flavor profile similar to that of the original cocoa butter. The isolated VFC were washed and collected by the use of diethyl ether, separated into basic, neutral and acidic fractions, and concentrated by the use of an Oldershaw column and then by spinning band distillation. The concentrated basic VFC were repeatedly fractionated by the use of a gas chromatograph until the compounds were pure or relatively pure before using infrared and gas chromatograph/mass spectrometer for identification. A total of 58 VFC was identified in the basic fraction of the two cocoa butter samples. They include 35 pyrazines, 3 pyrroles, 4 furans, 6 alcohols, 4 aldehydes, and 6 other compounds. There were significant differences, quantitatively and qualitatively, between the VFC of the expelled and pressed cocoa butters. Trimethylpyrazine was found to be the highest in concentration in pressed cocoa butter and the amount was 14.6 times more than that of the expelled sample. Among the total of 35 pyrazines, 30 of them were found in the pressed cocoa butter, while only 10 were identified in the expelled cocoa butter.

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A COMPARISON OF THE VOLATILE FLAVOR COMPOUNDS IDENTIFIED IN EXPELLED AND PRESSED COCOA BUTTER. II. ACIDIC AND NEUTRAL COMPOUNDS. James T. Carlin, Ken N.S. Lee and Stephen S. Chang, Dept. of Food Science, Cook College, Rutgers State University, P.O. Box 231, New Brunswick, NJ 08803.

The volatile flavor constituents of two cocoa butter samples with distinctly different aroma characteristics were compared. Cocoa butter obtained from roasted beans has a strong aroma which is reminiscent of cocoa, while that obtained from unroasted beans which are given a steam treatment is mild with acidic and floral characteristics. The volatile flavor constituents of both samples were isolated using an apparatus which employs continuous countercurrent vacuum steam distillation. The aqueous volatile solutions obtained were extracted with ether and separated into acidic, basic and neutral fractions. The acidic and neutral fractions were subjected to a systematic method of gas chromatographic fractionation in order to obtain pure compounds. The identities of the pure compounds were determined using infrared and mass spectroscopies and their relative abundances were calculated. A total of nine compounds was identified in the acidic fractions and 160 in the neutral fractions. There were significant quantitative differences between the two acidic fractions; however, no qualitative differences were found. The sample obtained from unroasted beans contained larger amounts of volatile fatty acids than did the sample from the roasted beans. The most dramatic difference was found for pentanoic acid where there was a twnety-three fold increase for the sample obtained from the unroasted beans. Both quantitative and qualitative differences were found between the neutral cocoa butter fractions. A series of pyrrole and furan compounds was identified in both samples but existed in larger quantities in the roasted sample. The compound, 5-methyl-2-phenylhexenal, has a chocolate-like aroma and was identified in large quantities in the roasted sample but was not identified in the unroasted sample. By comparing the relative abundances of the compounds identified in each sample the chemical changes that occur during the roasting process were characterized.

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EFFECT OF POLAR MINOR CONSTITUENTS ON THE FLAVOR STABILITY OF SOYBEAN OIL. J.L. Williams and R.G. Krishnamurthy, Kraft, Inc., 801 Waukegan Rd., Glenview, IL 60025.

A procedure for isolation of neutral triglycerides and polar compounds from soybean oil will be outlined. The effect of these polar compounds on the photosensitization of soybean oil triglycerides will be discussed. Changes in the volatile profiles induced by these polar compounds will be described. Significant differences in the light stability of neutral triglycerides with and without these polar compounds have been observed.

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PROGRESS REPORT OF THE AOCS FLAVOR NOMENCLATURE AND STANDARDS SUB-COMMITTEE. Arthur E. Waltking, CPC International Inc., Best Foods Research and Engineering Center, Box 1534, Union, NJ 07083.

Over the past four years, two collaborative studies have been organized to evaluate the GLC methods for flavor of edible oils. The most recent of these studies compared the results from five GLC procedures to the evaluations of the flavor panels of eight laboratories. While the GLC procedures proved to be more precise than the panels, it was shown that separate correlation equations must be developed for each type of oil, each degree of hydrogenation or blending, and each manner of storage or abuse of the samples. Thus, either a flavor panel must always be available to establish a reference point for any study to be made, or a method for GLC flavor be established to provide a direct but relative evaluation for "oil quality."

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THE USAFSAM CARDIOVASCULAR DISEASE FOLLOW-UP STUDY: CHOLESTEROL AND HDL LEVELS IN SUBJECTS WITH MYOCARDIAL INFRACTION. Dale A. Clark, Fred H. Wilson and Joel Michalek, U.S. Air Force School of Aerospace Medicine/NGP, Brooks AFB, TX 78235.

The USAFSAM Cardiovascular Disease Follow-up Study (West Point of '56) was begun in 1952 to accumulate information on the usefulness of lipid and lipoprotein levels measured in young men to predict risk of cardiovascular disease in their later years. Of the 474 original men, 424 are still active; 9 survived myocardial infarction (MI). The high density lipoprotein (HDL) and the cholesterol levels in the 9 MI survivors was compared with the levels in the 22 men having the highest 1% and in the 15 men with the lowest 1% of cholesterol levels at one or more times during the study. The data show (a) the average cholesterol level in the top 1% group is higher than that of either the lowest 1% or the MI group, and (b) the top 1% and the lowest 1% cholesterol groups do not differ significantly in mean HDL levels, but both are higher than the MI group. Since only 2 members of the MI group are also members of the top 1% group, elevated cholesterol alone does not identify individuals who will suffer myocardial infarction. The lower mean HDL level found in the MI group suggests that the risk associated with elevated cholesterol levels is significantly increased if the HDL level is decreased. While the average data are consistent with the view that men with elevated cholesterol levels may be spared from myocardial infarction by high levels of HDL, there are notable exceptions to that generalization.

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EFFECTS OF DIETARY FATS AND PHYSICAL TRAINING ON CARDIAC PHOSPHOLIPIDS IN RATS. G. Rocquelin and P. Juaneda, Institut National de la Recherche Agronomique, 17 Rue Sully, 21034 Dijon Cedex, France.

Cardiac phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), sphingomyelin (SM) and other minor phospholipids have been analyzed in trained and untrained male Wistar rats fed semi synthetic diet with 15% by weight of sunflower (SF) oil, high erucic acid rapeseed (HEAR) oil, low erucic

acid rapeseed (LEAR) oil, for 12 weeks. If compared to SF, LEAR as HEAR induces an increase of the cardiac DPG and SM contents (mg/g of organ) and a decrease of the PC and PE contents in untrained rats. Marked changes in the fatty acid spectra of these phospholipids are also observed. Cardiac PE and PC in trained and untrained rats fed LEAR or HEAR contain an elevated level of (n-3) C22:5 and C22:6 fatty acids whereas the (n-6) C22:4 and C22:5 disappear. Linoleic acid decreases in DPG of rats fed the cruciferous oils whereas (n-9) C18:1 increases. Levels of saturated C22, C24 fatty acids in SM of untrained rats fed LEAR and HEAR are lower than in animals fed SF. They are replaced by shorter saturated chains (C16, C18) or monoenes (C18, C20, C22, C24). These changes have to be related to the fatty acid composition of the cruciferous oils and particularly to their high level of monoenes (oleic or eicosenoic and docosenoic acids) and high ratio of linolenic acid/linoleic acid. Their possible implications in the structure and function of the cardiac membrane systems will be discussed.

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COMPARATIVE STUDIES OF THE CARDIAC LIPIDS OF RATS FED DIFFERENT VEGETABLE OILS. J.K.G. Kramer, Animal Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada, K1A 0C6.

The cardiac lipids of male rats fed various vegetable oils have been investigated to determine whether myocardial necrosis was related to change in the cardiac lipid classes or their fatty acid composition. Only minor changes occurred in the quantitative composition of both cardiac neutral- and phospholipids as a result of different dietary fatty acids. On the other hand, significant changes occurred in all the major cardiac lipids as a result of certain dietary fatty acids, particularly oleic, linoleic and linolenic acids. Dietary oleic acid affects specifically cardiac sphingomyelin and cardiolipin, while dietary linoleic and linolenic acids had a dramatic effect on the C22 polyunsaturated fatty acids of phosphatidylethanolamine and phosphatidylcholine. Despite these changes, a regulation of fatty acid composition was evident within the cardiac phospholipids presumably to maintain a structural integrity of cardiac membranes. The possible role of cardiac lipid changes to myocardial necrosis will be discussed.

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GLYCERIDE COMPOSITION INFLUENCE ON SERUM CHOLESTEROL AND SERUM HDL CHOLESTEROL LEVELS IN MAN. A. Christophe and G. Verdonk, Laboratory for Gerontology, Dietetics and Nutrition Research of the State University of Gent, Pasteurlaan 2, B 9000 Gent, Belgium.

In a first group of subjects, substitution of a triglyceride mixture rich in linoleic acid for butter resulted, after two weeks, in a decrease in total cholesterol (chol) which was mainly due to a decrease in non-HDL chol, HDL chol remaining constant. When this triglyceride mixture was replaced by a monoglyceride mixture (mainly DL α isomers) with the same fatty acid composition, there was a further decrease in total chol. In this instance however, it was mainly HDL-chol that decreased. Results will be presented that suggest that the lowering of HDL-chol levels when monoglycerides are substituted for triglycerides could be due to reduced lymphatic fat transport. In a second group of subjects, substitution of randomized butterfat for butter in the diet resulted, after 2 weeks, in a decrease in total chol without an effect on the HDL chol/total chol ratio. In a third experiment, linoleic acid rich monoglycerides were substituted for medium chain triglycerides. This was found to result in a decrease of both total and HDL chol without an effect on the HDL chol/total chol ratio. Neither in this case, nor in the former one, could reduction of HDL-chol be ascribed to reduced lymphatic fat transport, indicating that other mechanisms must also be involved. The results obtained demonstrate that the glyceride composition of a food fat, as well as its fatty acid composition, determines circulating levels of total chol and of HDL chol.

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CHANGES OF THE CONCENTRATION OF SERUM TOTAL CHOLESTEROL AND HDL-CHOLESTEROL DURING STARVATION IN OBESE SUBJECTS. A. Christophe and G. Verdonk, Laboratory for Gerontology, Dietetics and Nutrition Research of the State University of Gent, Pasteurlaan 2, B 9000 Gent, Belgium.

A group of 11 obese subjects (nine women; two men; relative body weight between 140% and 190%, normal mean 161%) underwent a therapeutic fasting treatment. Such a treatment consists of an initial caloric and sodium restriction (IR) period (2500 KJ; 400 mg sodium) of one week, followed by a total starvation (TS) period of three weeks (only water ad libitum) followed by a readaptation period of a few days of low caloric intake (2500 KJ and 400 mg sodium per day). Blood was taken before the treatment, after four days and at the end of the IR period, after 4, 10 and 21 days of TS and after 2 and 7 days of low caloric refeeding. After clotting overnight at 4 C, HDL-cholesterol was determined on the supernatant after dextrane sulphate-$CaCl_2$ precipitation of VLDL and LDL. During initial caloric restriction, there is a transient increase (in 10 cases out of 11; 10/11) in total cholesterol followed by a continuous decrease during the TS period (11/11). After a few days of low caloric refeeding, total cholesterol increases (5/11 after 2 days; 8/11 after 7 days), remaining lower after 7 days of refeeding than before the treatment. HDL chol, on the contrary, decreases immediately after institution of the treatment (8/11 after 4 days of caloric restriction; 11/11 after 4 days of TS) whereafter its further decrease is not much pronounced. Whereas the 2500 KJ diet resulted in a sharp decrease of HDL-chol before TS, it results in an increase after TS. The non-parallelism in changes of total cholesterol and of HDL-chol during the treatment results initially in a reduction of the fraction of the total cholesterol carried in the HDL and later in an increased HDL chol/total cholesterol ratio. These results will be related to changes in serum lipid and lipoproteins concentrations and changes in lipid metabolism known to occur during starvation.

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COMPARATIVE STUDY OF TRIACYLGLYCEROLS OF VERY LOW DENSITY LIPOPROTEINS (VLDL) OF NORMAL SUBJECTS AND PATIENTS WITH HYPERLIPEMIA. J.J. Myher, A. Kuksis, W.C. Breckenridge and J.A. Little, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6.

In a comparative study VLDL triacylglycerols were analyzed in individual and in pooled samples from normal subjects and patients with various types (Frederickson's) of hyperlipoproteinemia. VLDL was obtained from fasting subjects by conventional ultracentrifugation. Representative sn-1,2- and sn-2,3-diacylglycerols, generated by a partial Grignard degradation of the triacylglycerols, were stereospecifically resolved by phospholipase C via intermediate rac-1,2-diacylglycerol-3-phosphorylcholines. Molecular species of diacylglycerols were identified and quantitated by gas chromatography -mass spectrometry of the tertiary-butyldimethylsilyl ethers. The results of the stereospecific analyses demonstrated a marked asymmetry in the positional distribution of the fatty acids, with the saturated acids predominantly in the sn-1-position and the unsaturated fatty acids about equally distributed between the sen-2- and the sen-3-positions. Within the limits of experimental error the molecular species composition of the sn-1,2- and the sn-2,3-diacylglycerols was found to be identical to the composition calculated from the stereospecific distribution of the fatty acids assuming 1-random 2-random 3-random distribution. These findings suggest that the fatty acids in many instances are esterified to the triacylglycerol molecule completely independently of the composition of the acids in any other position, which would correspond to the noncorrelative distribution proposed earlier for hepatic triacylglycerols. As a measure of deviation from 1-random 2-random 3-random structure we propose an index of non-randomness (INR) defined as: $INR = (EXPT - CALC)^2$, where EXPT and CALC are the experimental and calculated compositons, and the summation is made over the entire series of molecular species. Duplicate analyses of the same triacylglycerol sample yield INR values ranging from 10-20, while non-random distributions give INR values one or two orders of magnitude higher, when compared to the corresponding random distributions. The hypothesis of non-correlative distribution of fatty acids in triacylglycerols is being assessed in other lipoprotein classes and under a variety of metabolic and physiological stimuli. (Supported by Ontario Heart Foundation, Medical Research Council of Canada and NIHNHLI-72-917, Bethesda, MD.)

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REGULATION OF HEPATIC LIPOGENESIS BY POLYUNSATURATED DIET FAT. Barbara C. O'Brien, Texas Agricultural Experiment Station, Consumer Research Center, Texas A&M University, College Station, TX 77843, and Raymond Reiser, Texas A/M University.

Short term control of lipogenesis by polyunsaturated diet fat (PUFA) has been demonstrated. Hepatic lipogenesis was stimulated by feeding a high sucrose fat-free diet to rats trained to consume a daily one-hour meal. Four hours after ingestion of a single meal containing 5% safflower oil (3.5% linoleate in the diet), incorporations of acetate and water into fatty acids by liver slices were suppressed compared to incorporations by livers from rats fed the fat-free meal or a single meal containing 5% cocoa butter (0.25% linoleate in the diet). The long-term regulation of hepatic lipogensis by PUFA which is characterized by attenuated lipogenic enzyme activities was not significantly affected based on fatty acid synthetase activities when prostaglandin synthase was inhibited by the administration of either aspirin or indomethacin. It appears that insulin may be involved in the long term control of hepatic lipogenesis by PUFA. Liver mitochondria from rats fed 5% safflower oil are as capable of supporting fatty acid biosynthesis from alanine in a cell-free lipogenic system as mitochondria from rats fed the fat-free diet or the diet containing 5% cocoa butter.

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THE DIETARY CONTROL OF HEPATIC LIPOGENESIS BY POLYUNSATURATED FATTY ACIDS. R. Jeffcoat and A.T. James, Unilever Research, Colworth Laboratory, Unilever Ltd., Colworth House, Sharnbrook, Bedford MK 44 1LQ, England.

When the energy intake of mammals exceeds the energy expenditure, the excess carbon is converted to triacylglycerol and stored in the adipose tissue. The major site of lipogenesis appears to vary from species to species and to be dependent upon the type of both dietary carbohydrate and fat. In man, as well as the experimental rat, dietary sucrose stimulates hepatic lipogenesis resulting in elevated plasma triacylglycerol and cholesterol. In the rat it has been demonstrated that fructose induces the synthesis of lipogenic enzymes and, in particular, fatty acid synthetase, the activity of which has been shown to be proportional to very low density lipoprotein secretion by the liver. In an attempt to understand more fully the dietary control of hepatic lipogenesis, we fed rats a range of carbohydrates and lipids. The results of these studies indicated that sucrose enhanced the activity of both fatty acid synthetase and stearoyl-CoA desaturase. Furthermore, the inclusion of low levels of dietary corn oil or ethyl linoleate could reverse this effect. It was demonstrated however that fatty acid synthetase responded very much more slowly than did stearoyl-CoA desaturase. In terms of dietary control, it would thus appear from the above observations, the diurnal variation of the desaturase and the results indicating that the desaturase is the rate limiting step in the de novo synthesis of oleic acid, that this enzyme catalyses an important control Step. A decrease in desaturase activity relative to the synthetase results in the inhibition of synthesis by the action of saturated fatty acids on acetyl-CoA carboxylase. A decreased triacylglycerol synthesis results not only from this reduced fatty acid synthesis, but also from the relative decreased synthesis of oleic acid which compared to saturated fatty acid is the preferred substrate for triacylglycerol synthesis. A model for the control of hepatic lipogenesis by the action of linoleic acid on desaturation will be discussed in relation to the deposition of fat.

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THE USE OF MONOGLYCERIDE IN CHEMICALLY DEFINED DIETS. Dac Lekim and M. Stahl, LEPHARM GmbH., Escher Ringstr. 33, 5 Cologne 71, West Germany.

The protein component of chemically defined diets, particularly those for patients with pancreatic insufficiency or intestinal resorption difficulties, is usually hydrolyzed proteins, peptides or amino acids. The lipid component is in this case absent or composed of middle chain triglycerides with or without vegetable oil which is only digested with difficulties. Long chain monoglyceride is used mostly, up to now, as an emulsifier only. Liquid and powder chemically defined diets with long chain monoglyceride containing up to 10% of energy in the form of essential fatty acids are constituted according to the procedure to be described. The incorporation of monoglyceride in such diets is greatly aided by natural co-emulsifiers. This type of diet is shown to behave well in stability tests. In particular, the lipid fraction is analyzed by a combination of silicic column and HPLC. The acceptability and the resorption of such diets in proven in clinical studies. The results show that the monoglycerides are well taken up without side effects in normal patients and in patients with pancreatic deficiency.

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NUTRITIONAL QUALITY OF INTERESTERIFIED FAT PRODUCTS. D.K. Bhattacharyya, M.M. Chakrabarty and K. Kar, Department of Applied Chemistry, University Colleges of Science and Technology, 92 Acharyya Prafulla Chandra Road, Calcutta, 700009, India.

Much interest has grown of late in India in the use of nontraditional oils in preparing edible fat products by the interesterification process. Many nutrition experts feel hydrogenation may be undesirable. While the technology is now known in India for the production of *trans*-free PUFA-rich fat products from less known oils for use as margarines and vanaspati substitutes, the nutritional quality of such kind of fat products has not been investigated. In the present study, the nutritional quality of the fat products with melting range of 35–37 C having been prepared from mowrah *(Madhuca latifolia)*, rice bran *(Oryza sativa)* containing sal *(Shorea robusta)*, and cottonseed *(Gossypium hirsutum)* mixed with sal by randomization has been examined and compared with that of the vanaspati produced in India by hydrogenation of mixtures of liquid oils and used exclusively as a substitute of ghee (butter fat). The biochemical parameters examined after feeding the rats a diet containing the interesterified fat products are growth rate, relative organ weight, total lipid and lipid composition, such as the content of total cholesterol, free cholesterol, triglyceride and phospholipid of different tissues like liver, heart, serum and kidney, compared with those of the control rats fed vanaspati, the hydrogenated fat. The co-efficient of digestibility of the fats are determined to examine the use of these fats in the gastrointestinal tract. Experimental results confirm that the interesterified fat products can be suitably used as substitutes of vanaspati.

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THE BUFFALO GOURD, *CUCURBITA FOETIDISSIMA*, AS A SOURCE OF EDIBLE VEGETABLE OIL. W.P. Bemis, J.W. Berry, and C.W. Weber, Department of Plant Sciences, College of Agriculture, University of Arizona, Tucson, AZ 85721; and J.A. Vasconcellos, Universidad de las Americas.

The Buffalo Gourd, *Cucurbia foetidissima,* is a feral plant species which has evolved in the arid to semi-arid areas of western North America. It is a perennial vine and prolific producer of fruit which contains seed having 30–40% edible oil. This plant is currently the object of a domestication study at the University of Arizona. The principal fatty acid components of the oil are linoleic (58.2%), oleic (27.0%), palmitic (9.7%) and stearic (4.5%). Unrefined oil fed to weanling mice in isocaloric isonitrogenous diets of up to 11% of the diet being oil gave normal growth with no evidence of deleterious effects. The oil is essentially free of conjugated fatty acids, having about 2.5% dienes and only a trace of trienes. The crude oil has a relatively high carotenoid content (100 mg/kg). These and other impurities are removed by current refining methods. Summary: a perennial crop plant adapted to arid land agricultural produces an acceptable vegetable oil in addition to protein and starch in commercially acceptable quantities.

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SUNFLOWER–A NEW MAJOR OILSEED CROP IN THE UNITED STATES. Don C. Zimmerman, Agricultural Research, SEA-USDA, Dept. Biochemistry, North Dakota State University, Fargo, ND 58105.

The increase in sunflower production in the United States has increased dramatically in the last five years to the point where the United States is now the major exporter of sunflower seed in the world. The rapid increase in sunflower acreage has been greatly facilitated by the introduction of hybrid varieties with improved yield potential and disease resistance. Because of its profitability relative to other crops in the north central states, farmer acceptance has been very good. In the past, strong export demand was the major economic marketing factor for sunflower seed. However, a United States market for sunflower as an edible oil has increased the domestic crush of sunflower seed. With several new processing plants planned for construction close to the major production areas, there will be an increase in the production of sunflower oil for both domestic use and for export.

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PLANT BREEDING AND ENGINEERING RESEARCH FOR THE DEVELOPMENT OF SESAME AS A COMMERCIAL CROP. D.M. Yermanos, Dept. of Botany & Plant Sciences, University of California, Riverside, CA 92521.

The demand for sesame seed and oil in the U.S. has always been met through imports from Africa and Central America. The food industry has expressed interest in using sesame as a source of protein and edible oil. Scarcity and high cost of the seed has limited its use to mostly decorative applications. Sesame is not grown in the U.S. because of high labor requirements for harvesting. Indehiscent strains of sesame with desirable agronomic characteristics and improved yield potential are being developed at UCR. Suitable harvesting equipment has also been designed for threshing indehiscent strains of sesame. Thus, it is hoped that domestic production of sesame may commence in the near future.

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MANGO FAT. S.M. Osman, Department of Chemistry, Aligarh Muslim University, Aligarh-202001, India.

A number of workers in India have carried out intensive studies on the fat composition, protein content, and aroma and flavor constituents of mango *(Mangifera indica,* Anacardiaceae). The solid fat (5 to 15% depending upon variety) resembles cocoa butter and hence is a potential source of commercial oil. Data on varietal differences on characteristics of kernel fat indicate that the fatty acid composition is dominated by palmitic (6-18%), stearic (24-49%) and oleic (33-53%). The major glycerides are StOO (54%), POS (16%), OStSt (8%) and S3 (14%). Studies of dilatometric and cooling characteristics indicate that refined mango fat differs from cocoa fat. Pulp lipid compositional study of two commercial varieties of mango during ripening has indicated that in the case of Alphonso mango there is an increase in palmitoleic and linolenic acid contents. The ripening of this mango variety was found to be associated with concurrent changes in glyceride (mainly triglyceride, 55%) content and fatty acid composition. Pulp lipid biogenesis investigations suggest a correlation between the relative proportion of C-16 acids (palmitic and palmitoleic) with the fruit aroma and flavor. Examination of volatile constituents of the Egyptian mango showed the presence of lower alcohols and ethyl acetate. The deoiled cake constituting 90% is rich in edible starch and proteins

suitable for cattle and poultry feed. The only storage problem is the fat hydrolysis in high humidity conditions increasing the free fatty acid level up to 25%. Experiments on the processing of mango stones (seeds) for extracting fat suggest a great potential of mango fat for use as a cocoa fat substitute from fats of tree origin.

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LUPINUS MUTABILIS SWEET–A POTENTIAL FOOD OIL AND PROTEIN SOURCE. A.M. Davis, 59 Johnson Hall, Washington State University, Pullman, WA 99164.

Lupinus mutabilis Sweet, known as tarwi, tarhui, tarhui-chocho and many other local names, is a common food source in the Andean mountains of South America. It contains a bitter and toxic alkaloid which must be removed before eating. It is heat labile and water soluble. After removal of the alkaloids, the seeds are over 50% oil and protein (14–24% oil and 41 to 50% protein). The protein is deficient in only methionine, adequate in lysine and cystine and is as digestible as soybean protein. A report from Peru showed the average of 23 breeding lines was: lipids, 24.6%; protein, 43.8%; ash, 4.70%; fiber 9.50%; carbohydrates 17.80% and total alkaloids 1.66%. If an economical means of removing the alkaloids can be developed and breeding work can develop strains that are determinant in growth habit and adapted to temperate climates, tarwi could become a new food and oil source for many of the highly populated developing countries.

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LIPIDS FROM MICROORGANISMS, ESPECIALLY METHYLOMONAS CLARA. Merten Schlingmann, Hoechst AG, Zentralforschung II–Biotechnik/H 777, 6000 Frankfurty/M. 80 West Germany and Uwe Faust, UHDE GmbH.

An easy method for the extraction of fats from microorganisms as well as from other protein materials will be described, analytical data shown, and results discussed. One subject of our work is the development of food proteins based on crude proteins sources from microorganisms. By means of a newly developed, two-stage extraction of bioproteins products low in fats and nucleic acids can be produced. The first stage concerns the treatment of the cells with special organic solvent/nonaqueous ammonia mixtures. In such a way the lipids of the cell wall and membrane can be dissolved and isolated natively. The methanol utilizing bacterium Methylomonas clara contains 5-20% lipids depending on the fermentation conditions. The extracted and isolated lipids consist of 25% free fatty acids, 40-45% phospholipids and 25% polar lipids. 90% of the free and bound fatty acids are $C_{16:1}$ and C_{16}. The process can also be converted to other crude proteins from plants and animals like soy, lupines, fishmeal or milkpowder.

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UNUSUAL SEED OILS AND THEIR FATTY ACIDS. Cecil R. Smith Jr., Research Center, AR/SEA, USDA, 1815 N. University St., Peoria, IL 61604.

From 1958 onward, the Northern Regional Research Center has conducted a program of screening, characterization, and preliminary development aimed at establishment of new oilseed crops. Primary emphasis has been on presently uncultivated plants that provide nonedible oils for new end uses or markets. This paper will summarize the major discoveries and conclusions that have emerged from this program in terms of structure and chemistry of the unusual fatty acids produced in major proportions by these prospective oilseeds. Possible end uses will be considered, as well as quality of defatted seed meals and agronomic potential. Groups of oils or seed oil sources to be mentioned include: the genus *Lesquerella,* source of several new hydroxy fatty acids related to those in castor oil; *Dimorphotheca,* source of a highly reactive conjugated hydroxydienoic acid (dimorphecolic acid); *Crepis* species, several of which provide crepenynic acid, an acetylenic analogue of linoleic acid; *Limnanthes* seed oils, which have a uniquely high percentage of fatty acids longer than C_{18}; epoxy acid sources, including a botanically diverse group of plants; the genus *Cuphea,* whose seed oils produce a predominance of C_8, C_{10}, or C_{12} fatty acids, depending on the species; *Briza spicata,* a unique source of galactolipids; pertroselinic acid sources, mainly represented by the plant family Umbelliferae; and *Crambe,* source of high erucic acid oils. *Crambe, Limnanthes,* and some epoxy acid-producing oils will be discussed in more detail by other speakers.

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LIMNANTHES: CURRENT STATUS OF AGRONOMIC PRODUCTION. Gary D. Jolliff, Wheeler Calhoun and J.M. Crane, Crop Science Department, Corvallis, OR 97331.

The process of domestication of a wild plant usually involves changing the genetics of the plants for at least three major reasons: (a) adaptation to the environment where production is desired; (b) maximizing yield of the desired product; and (c) adaptation to the available production technology, such as mechanization. Native plants of meadowfoam *(Limnanthes alba)* are well adapted to the climate of the Willamette Valley of Oregon as shown by consistently good growth and development of the plant. Acceptable levels of the seed oil are produced. Genetic variability in oil quality provides some opportunity to meet varying demands which might develop in the future in the industrial oil market. Adaptation to the production technology and cropping systems of the Willamette Valley has been excellent. Meadowfoam is the only crop which has shown a potential as an alternative to growing annual ryegrass for seed on very poorly drained soils in the Willamette Valley. We have developed a new variety of meadowfoam which has an upright growth habit, improved seed retention and does not exhibit secondary seed dormancy under our growing conditions. This has allowed us to produce the crop on a commercial scale. Slides and data will used to illustrate the results of work with seed-bed preparation, planting, fertilization, weed control, harvesting, and processing.

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THE WILD CUCURBIT, *APODANTHERA UNDULATA,* AS A NEW PLANT SOURCE OF VEGETABLE OIL. W.P. Bemis, J.W. Berry and C.W. Weber, Department of Plant Science, College of Agriculture, University of Arizona, Tucson, AZ 85721, and J.A. Vasconcellos, Universidad de las Americas.

The wild cucurbit, *Apodanthera undulata,* is a perennial species indigenous to southwestern United States and northern Mexico. The plant is a dense prostrate vine producing many soft cucumber-like fruit 7-10 cm in length and 4-5 cm in width. The fruit are essentially seed balls containing about 100 seed per fruit weighing approximately 14 g per 100 seed. The seed contain 33% protein and 35% crude oil. The oil contains conjugated fatty acids, about 5% dienes and 15-20% trienes (punicic acid). These relative high levels of conjugated fatty acids and the high levels of acetyl and hydroxyl values, approximately 25% each, identify the oil as a drying oil rather than an edible oil.

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STOKESIA LAEVIS: A POTENTIAL NEW SOURCE OF EPOXY ACID. T.A. Campbell, USDA SEA,AR,NER,PGGI,GRL Bldg. 001, Room 340, BARC-W, Beltsville, MD 20705.

Stokesia Laevis (Hill) Greene is an erect, perennial composite native to the south-eastern United States. It has an achene oil content of up to 44%. Since as much as 79% of this oil is vernolic acid (*cis*-12,13-epxoy-*cis*-9-octadecenoic), *S. Laevis* has potential as a natural source of epoxy acid. Studies indicate the species is essentially cross-pollinated and that cross-fertilization by insects is necessary for good seed production. Preliminary seed yield estimates are as high as 1,160 kg/ha. Vernalization (cold treatment) for four to five months is necessary to induce flowering and flowering period is highly variable among accessions. Three major deterrents to agronomic development are seed dormancy, poor seedling vigor, and poor seed retention. Once established, plants are extremely vigorous with excellent stand longevity. There appear to be no major disease problems and preliminary herbicide studies indicate tolerance to a broad range of herbicides. Genetic variation is excellent in the species and prospects for developing agronomically desirable types are good.

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AN IMPROVED METHOD OF OILSEED EXTRACTION (ESPECIALLY FROM COTTONSEED). Dan Ram, Miloumor-Mobile, Post Ashrat, Israel.

The meats, with 16-19% moisture, are heated to 90-100 C, while passing through a kneader and a patented oil separator located coaxially with the kneader. This takes only 5-7 minutes and causes rupture of the pigment glands and release of oil and gossypol through the serrated bars of the separator. About 42-55% of the oil is separated at this stage. The meats leaving the separator are conveyed to a solvent extraction plant for extraction of the remaining oil. In order to realize the advantages of the presented method, let us compare it to the classical existing ones which may be classified as follows: (a) pressing of cooked meal by screw press; (b) direct solvent extraction after preliminary cooking; (c) combining the previous two processes. The cooking of the meats, an integral part of these methods, takes 40-70 minutes at a temperature of about 110 C. The comparatively lengthy heating is required to reduce the free gossypol, which is chemically combined with the lysine and to facilitate the release of the oil. Thus lysine in the meal is reduced and also the solubility of the protein. The crew presses require a high power input and its screw is exposed to rapid wear, which involves expensive maintenance. Thus a method with minimum heating and lower pressure would be of great advantage. The presented method replaces the prepressing stage of method No. 3 mentioned above. It requires lower temperature for a short period (5-7 min), thereby improving the solubility of the proteins in the meal and the quality of oil. The meal is also considerably richer with lysine since most of the gossypol is readily carried with the oil.

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A PILOT PLANT AND LABORATORY STUDY OF THE DE-

SOLVENTIZATION OF CANOLA MEALS. John A. Blake and Myles N. Marianchuk, POS Pilot Plant Corp., U of S Campus, Saskatoon, Saskatchewan S7N 2R4 Canada.

In laboratory studies, ammonia was compared to nitrogen as sparging gas in the desolventization of hexane-laden Canola meals. Ammonia was somewhat better than nitrogen, but not sufficiently so to warrant its use strictly as a sparging gas. The color and odor of the ammonia treated meals were considerably improved, however. The glucosinolate content of either ammonia or nitrogen treated meals was not altered even at desolventization temperatures of 100 C. Pilot plant studies have shown that at a desolventizer retention time of one hour, sparge steam may be omitted or greatly reduced in the desolventization of rapeseed meal. Residual solvent levels attained were of the order of 550 ppm hexane.

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PROPERTIES OF PEANUT PROTEINS FOR FOOD APPLICATION. Robert L. Ory and Edith J. Conkerton, Southern Regional Research Center, USDA, SEA, P.O. Box 19687, 1100 Robert E. Lee Blvd., New Orleans, LA 70179.

Peanuts contain 50-55% oil and 27-30% protein. Comparison of essential amino acid compositions of commercially available red-skin peanuts and bland-flavored white-skin peanuts (under investigation as a new source of vegetable protein) showed essentially the same profiles. Lysine and methionine are the most limiting in the varieties examined. Blanching of white-skin peanuts is not required for preparation of oil-free high-protein flours. Processing costs are thus lower and fiber content higher, but a white flour is still produced. Methods for improving nutritional properties of peanut flour by blending with other plant proteins were investigated. Methionine, lysine, and chemical scores of peanut flour are improved by such blends. Because of the high solubility of peanut proteins, however, solubility properties of blends are not improved. The lowered solubility might limit the use of blends in beverage-type products but should not affect utilization of peanut protein blends in solid foods, such as bakery items or extended meat products.

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AMARANTH GRAIN: A POTENTIAL SOURCE OF HIGH QUALITY FOOD PROTEIN. Joseph P. Senft and Charles S. Kauffman, Organic Gardening and Farming Research Center, Rodale Press, Inc., P.O. Box, 323/RD #1; Kutztown, PA 19530.

Amaranth grain was an important staple of early South American cultures. In 1975, a National Academy of Sciences panel identified grain amaranth as "an underexploited plant with promising economic value." Initial selections from a worldwide germplasm collection of over 500 accessions have been made. Higher yielding selections have produced as much as 2000 kg/hectare for a temperate climate. Proximate analysis shows 16% protein, 7% lipid, 60% carbohydrate, 2.5% fiber, 2.6% ash, 12% moisture. Protein quality is high; lysine 5.0%, methionine-cysteic acid, 4.4%. Leucine, 4.7%, is the limiting amino acid. Animal feeding experiments have given protein efficiency ratios of 1.5 to 2.2. In some feeding studies, a heat labile substance has had an adverse effect on palatability. Oxalate content if 0.4% of which one third is soluable or "available" oxalate. The significance of oxalate content to nutritional quality will be discussed.

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EFFECT OF SUCCINYLATION OF COTTONSEED PROTEINS DURING EXTRACTION ON THE YIELD AND CHEMICAL AND FUNCTIONAL PROPERTIES OF ISOLATES. Y.R. Choi, E.W. Lusas and K.C. Rhee, Food Protein Research & Development Center, F.E. Box 183, Texas A&M University, College Station, TX 77843.

Treatment of defatted cottonseed flour with succinyl anhydride during aqueous extraction produced protein ingredients with highly desirable functional properties for formulation of diary analogs. Relationships between the extent of succinylation and extractibility and recovery of protein, and changes in protein solubilities and molecular size distribution, were studied. Also, chemical and functional properties of the modified protein were determined. Succinylation significantly increases extractability and recovery of cottonseed protein over the conventional method or the water extraction method alone. This modification resulted in conversion of water-insoluble proteins (such as salt- and/or alkali-soluble proteins) to water-soluble forms. The resulting products had larger molecular sizes, and showed higher water solubility, less calcium precipitability, less heat coagulation, and higher emulsion capacity and stability than water- or salt-extracted products. Succinylation also increased efficiency of centrifugal recovery of proteins by formation of larger floccules during isoelectric precipitation.

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UTILIZATION OF MEMBRANE-PRODUCED OILSEED ISOLATES IN FROZEN DESSERTS. J.T. Lawhon, N.H. Golightly and E.W. Lusas, Food Protein Research & Development Center, F.E. Box 183, Texas A&M University, College Station, TX 77843.

Consumption of frozen desserts in the United States has increased steadily in recent years. However, rising costs of milk solids-not-fat (MSNF) used in dessert formulas are causing manufacturers to consider less-expensive nondairy protein sources as an alternative. Use of soy protein isolates and concentrates as food ingredients is rapidly gained acceptance in the United States. Glandless cottonseed and peanut protein isolates are expected to become available in the next few years. Investigators at Texas A&M University's Food Protein Research and Development Center have developed a membrane isolation process (MIP) which employs ultrafiltration membranes to produce protein isolates directly from oilseed flour extracts. MIP isolates possess functional and nutritional properties that differ from conventionally produced isolates. Their performance in frozen desserts was assessed by replacing protein from MSNF at four levels (20%, 40%, 60% and 80%) with each of three oilseed isolates (soy, glandless cottonseed storage protein, and peanut). Taste panel scores of dessert samples for color, odor, texture, flavor and overall acceptability were statistically analyzed. Results showed that up to 40% of the MSNF could be replaced with MIP soy or peanut isolates without loss of color lightness. No textural changes occurred with soy or peanut replacements through the 80% level. Cottonseed isolate affected texture adversely beyond the 40% replacement level. Dessert flavor was not affected by using soy through the 80% replacement level. However, peanut and cottonseed isolates lowered flavor scores beyond the 60% level. Samples containing soy and peanut isolates at levels through 60% did not differ in overall acceptability from a 0% replacement control sample. The cottonseed storage protein isolate did not affect overall acceptability at the 20% replacement level. Based on these results, MIP oilseed isolates are a viable alternate source of protein for use in frozen desserts to the replacement levels stipulated.

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SOME TECHNOLOGICAL ASPECTS OF THE PREPARATION OF SUNFLOWER PROTEIN CONCENTRATES. Janos Stroszel and Jelena Dominez, Institute of Food Science and Technology, Jagićeva 31, 41000 Zagreb, Yugoslavia.

In Yugoslavia, as in many areas of the world, high-oil content sunflower hybrids have been used as sources of edible oil and sources of crude protein for animal feed. Substantial change of technological qualities of introduced hybrids requires fundamental changes in processing technology. It will be necessary to find new ways of dehulling, oil extraction, oil refinement and recovery of proteins in processing of seed for production good quality oil and potentially food grade protein. The purpose of the study was to find out a solvent system for optimizing chlorogenic acid removal and their selection toward lowering denaturation effect. We studied only solvents and solvent mixtures which we considered suitable for extraction of meats and meals on industrial scale. In our lecture we shall discuss our findings on chlorogenic acid binding, removal and effects of azeotropic mixtures extraction on protein solubility in the meats and meals. We also studied the behaviour of hydrophylic cytoplasmic components (protein bodies) during extraction by scanning electron microscopy. Results of extraction of dehulled sunflower meats and re-extracted meals with azeotropic mixtures, showed that some of these mixtures gave concentrates with good protein solubility and acceptable color, flavour and some functional properties, better than concentrates obtained by diffusion extraction method.

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PROTEIN QUALITY OF PEANUTS AS INFLUENCED BY CULTIVAR AND DIFFERENCES IN PROTEIN QUALITY OF PEANUTS ASSOCIATED WITH CULTIVAR AND LOCATION OF PRODUCTION. Josephine Miller, Department of Food Science, University of Georgia College of Agric. Experiment Station, Georgia Station, Experiment, GA 30212, and T.H. Sanders, National Peanut Research Lab.

Four cultivars of peanuts (Florigiant, Starr, Tamnut and Florunner) grown at two locations (Raleigh, NC, and Stephenville, TX) as part of the national variety trials were evaluated for protein nutritional quality by rat protein efficiency ratio (PER) bioassay. Peanuts of the 1978 crop were blanched with a minimum of heat treatment, and approximately half of the oil of the peanuts was expressed on a Carver press. The partially defatted peanuts were ground, packed into glass columns and extracted with hexane. The meals were incorporated into rat diets as the sole source of protein and provided 10% protein (Kjeldahl nitrogen x 6.25) by weight. The diets contained a complete complement of vitamins, minerals, and essential fatty acids for growth of weanling rats. A group of 10 rats was fed diets containing each of the eight meals and one containing casein for 28 days. The rats were housed individually, and provided with food and deionized water ad libitum. PER was calculated as the ratio of weight gained to protein consumed for th 28-day period. PER values of the eight peanut meals ranged from 1.7 to 1.3 compared to an adjusted PER value of 2.5 for the casein control

diet. There were no statistically significant differences in PER among the four cultivars grown in Raleigh. PER of the meal from three of the cultivars grown in Stephenville was significantly lower than that of the same cultivar produced in Raleigh. (Starr cultivar was not different between locations.) Among the four cultivars produced at Stephenville, PER of Tamnut and Florunner was significantly lower than that of Starr and Florigiant. Further studies are under way to determine if amino acid supplementation will influence the differences in PER associated with location of production.

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SEED PROTEINS IN CULTIVATED PEANUTS: AN IMMUNOCHEMICAL/ELECTROPHORETIC STUDY. Navin J. Neucere, USDA, SEA-AR, Southern Regional Research Center, P.O. Box 19687, 1100 Robert E. Lee Blvd., New Orleans, LA 70179.

Disc electrophoresis of proteins in several varieties of Virginia and Spanish-type peanuts showed very similar profiles with minor differences in histochemical staining and electrophoretic mobilities. Analysis by immunoabsorption showed identical composition of major proteins in all varieties of market-type peanuts. Immunoelectrophoresis of the classic conarachin system with diverse immune sera, however, showed slight differences in precipitin profiles. Chromatographic properties of total proteins in two varieties differed in elution patterns, primarily in the proportional concentrations of albumins and conarachins.

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SOY PROTEIN CONCENTRATE BY EXTRACTION WITH AQUEOUS ALCOHOLS. George Karnofsky, Dravo Corporation, One Oliver Plaza, Pittsburgh, PA 15222.

A process is described by which soy protein concentrate or flour is made by a solvent extraction process employing aqueous ethanol solutions as the only solvents. Dehulled full-fat flakes are extracted first with 50-70% ethanol to remove carbohydrates and most of the lipids, then with hot 90-95% alcohol to remove oil. This is accomplished in sequential countercurrent steps. In Step II dilute alcohol is displaced, from the flakes extracted in Step I, by strong alcohol from Step III. In Step III the flakes are extracted with recycling strong alcohol; the miscella is cooled to precipitate oil; and a portion of the cooled miscella is advanced to Step II while the remainder is reheated and recycled to the extractor. In Step IV the last of the oil is extracted with fresh strong alcohol which advances to Step III. The products are essentially tasteless protein concentrate, very low in lipids, and oil which is pale yellow and free of break. Experimental methods are described, and typical data included.

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ADSORPTIVE REFINING OF EDIBLE OILS. A.K. Sen Gupta, Unilever Forschungsgesellschaft mbH, Behringstr. 154, D-2000 Hamburg, West Germany.

Use of adsorbents in the refining of edible oils is reviewed. After the consideration of different theoretical aspects connected with adsorption processes, different types of adsorbents, process conditions under which these are used and the effects achieved are described. Distinction is made between slurry process and fixed bed process. Advantages and disadvantages of fixed bed and slurry processes are discussed with particular reference to the use of adsorbent with or without solvent. Influence of pore characteristics, acidity and other parameters of the adsorbents on the removal of phospholipids, color bodies, polar and other minor components of oils are discussed on the basis of own experimental results. The suitability of various adsorptive processes for industrial applications is discussed.

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USE OF ACTIVATED CARBON FOR REFINIING OF EDIBLE OILS. Hans H.R.H. Wendt, Union Deutsche Lebensmittelwerke GmbH, Zweigniederlassung Hamburg-Bahrenfeld, Friedensallee 333, D-2000 Hamburg 50, Federal Republic of Germany.

After publication in 1968 by Grimmer of a list of 13 polyaromatic hydrocarbons found in ppb-amounts in some crude oils, experiments were carried out how to remove these compounds. It was found that treatment with activated carbon, in combination with deodorization, was very efficient. The carbon is added during the bleaching stage, after addition of bleaching earth. The amount necessary varies between 0.4% for heavily contaminated oils, such as coconut oil, and 0.25% for less heavily contaminated oils, such as, e.g., sunflowerseed oil. Contamination of vegetable oils by polyaromatic hydrocarbons is caused by smoke-drying of oilseeds. This is common practice in the eastern hemisphere, while in the western half of the globe oilseeds are air-dried, and consequently will be free from polyaromatic hydrocarbons. Analytical determination of polyaromatic hydrocarbons in oils is still very time-consuming. We are using a modification of the original Grimmer method, which has been developed by Unilever Research in Hamburg. Other methods have been proposed by several authors. In the absence of official maximum levels, Unilever has fixed internal standards, limiting the total of polyaromatic hydrocarbons in the ready products. This is checked as a routine-analysis for all brands manufactured by the German Unilever margarine factories.

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SOME ASPECTS ON ADSORBENT TREATMENT AND HYDROGENATION OF FISH OIL. Ernst H. Goebel, Quimica Sumex, S.A. de C.V., Apartado Postal 19-201, Mexico 19, DF, Mexico, and Miguel Romero, Investigation Y Desarrollo Industrial, S.A.

Since crude fish oil contains a large variety of undesirable toxic metal traces and catalyst poisoning impurities, adsorbent treatments before hydrogenation are being discussed. The effects of such treatments on hydrogenation efficiency and catalyst performance are explored for several types of hydrogenation, including selective, nonselective and touch hydrogenation. Results of introductory tests on crude and refined oils with respect to changes of metal traces measured by atomic absorption spectrophotometry will be presented. As sulfur is the catalyst poison most frequently found in measurable amounts in fish oil, main emphasis is placed upon sulfur removal from the fish oil to be hydrogenated. Several adsorbents are evaluated.

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CONTROL OF FREE FATTY ACID RISE IN CLAY-TREATED VEGETABLE OILS. Dennis R. Taylor, Benjamin A. Root and Charles B. Ungermann, Filtrol Corp., 3250 E. Washington Blvd., Los Angeles, CA 90023.

Contacting raw vegetable oils at about 220-250° with acid-activated clays at low level (0.25-2.0 wt %) is an efficient and time-honored method for bleaching color bodies from edible oils. However, one undesirable consequence of this operation under certain circumstances is that the free-fatty acid content of the treated oil is increased. New methods of controlling free fatty acid rise during bleaching operations are discussed. Data will be presented from laboratory and commercial tests.

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OIL RECOVERY FROM BLEACHING CLAY BY A THREE-PHASE EXTRACTION AND SEPARATION COLUMN. Klaus Weber, Extraktionstechnik GmbH Postfach 76 01 47, D-2000 Hamburg 76.

The disposal of spent bleaching clay is creating environmental and economic problems. The different technologies in use to recover oil from bleaching clay are not completely satisfying in several aspects. An attempt was made to overcome some deficiencies of the existing recovery systems by developing a new technology, based on the easy separation by gravity of a nonpolar solvent, water and bleaching earth in a column. The particular behavior of the components involved in this process allows a simple removal of the oil from the earth particle by action of the solvent and a subsequent displacement of the adhering solvent by water. The low investment and operational costs which this new process offers, gives a new incentive to reconsider the handling of spent bleaching clay.

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OIL REMOVAL AT MUNICIPAL WASTEWATER TREATMENT PLANTS. G.N. McDermott, The Procter & Gamble Company, 6105 Center Hill Rd., Cincinnati, OH 45224.

Municipalities have traditionally limited the concentrations of oil of animal as vegetable origin in process wastewaters discharged to municipal systems. One reason for doing so is the claim that such oils interfere with the normal operating of the plants and cause the plant to violate standards for its effluent quality. The performance of municipal plants in removal of oil and grease of all types has only recently been reevaluated based on a compilation of available data from municipalities. The EPA in October 1979 reported limited data it had obtained from a survey of municipal plant performance. Additional data gathering is planned by U.S. EPA. This data indicates the effectiveness of standard biological treatment processes in oil removal is equal or superior to the removal of other organic matter in domestic sewage. This data further supports the contention that limitations on dispersed animal or vegetable oil are not needed. Such restrictions should be eliminated so that the economies of scale achieved by joint treatment are not lost to society.

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ENVIRONMENTAL REGULATIONS IN EUROPEAN COUNTRIES. Gustaf Blidberg, IVL-Consulting Ltd., Box 21060, S-100 31 Stockholm, Sweden.

The current and anticipated environmental regulations for the edible oil and fatty chemical plants in Scandinavia and some major European countries will be presented and discussed. Aspects will be given on air and water pollution problems as well as handling of solid waste within this industry. General technical solutions for

pollution control used by the industry in the different countries to cope with their present regulations will be briefly discussed as well as anticipated areas for future development.

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CRITICAL SAFETY DEVICES. Harold Sandvig, Department 2, P.O. Box 9300, Minneapolis, MN 55440.

This talk will concern itself with mechanical, electrical and electronic systems adjunct to the safe operation of a solvent extraction processing plant. Included will be a discussion of the needs, applications and desired results of these installations.

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DUST EXPLOSIONS IN OILSEED PLANTS. Ing. Radant, Berufsgenossenschaft Nahrungsmittel und Gastatten, Steubenstrasse, 46, 6800 Mannheim 1, West Germany.

This paper will discuss the hazards of dust explosion in oilseed processing plants and storage facilities and methods used to eliminate such risks. The data presented is from Germany, and the regulations required in that country in building such facilities will be explained. A 20-minute film will be shown after the talk is presented.

447

EXTRACTION PLANT PURGING. Marvin Woods, Lauhoff Grain Co., 329 E. North Street, Danville, IL 61832.

Purging solvent extraction plants will be reviewed for plant start up and shutdown during emergency conditions, and for complete shutdown of the plant for welding. Different methods of purging will also be reviewed, and this will include the use of air, steam and inert gas.

448

EXPLANATION, QUESTIONS, AND DISCUSSION OF BOOKLET, NFPA NO. 36, SOLVENT EXTRACTION PLANTS. C. Louis Kingsbaker, De Smet U.S.A. Corp., 2625 Cumberland Parkway, Suite 200, Atlanta, GA 30339.

A brief discussion of the most important rules stated in NFPA No. 36 will be presented along with the recent changes made in the 1978 edition. The distance diagram will be explained.

449

BUTYDIMETHYLSILYL ETHERS OF HYDROXY ESTERS AND IODINE-CATALYZED SOLVOLYSIS PRODUCTS OF LONG-CHAIN EPOXIDES. D.E. Minnikin and S.M. Minnikin, Department of Organic Chemistry, The University of Newcastle upon Tyne NE1 7RU, Great Britain.

Trimethylsilyl ethers have been used very successfully as derivatives in the gas chromatography and mass spectrometry of long-chain hydroxy compounds particularly those derived from unsaturated fatty acids (for a review see Minnikin, Chem. Phys. Lipids 1989, 21:313). The greatly increased resistance to hydrolysis of *t*-butyldimethylsilyl (TBDMS) ethers (Corey and Venkateswarlu, J. Amer. Chem. Soc. 1972, 94 6190) suggested that these derivatives might be useful since purification by thin-layer chromatography would be possible. In a recent communication (Minnikin and Patel, Chem. Phys. Lipids 1979, 23:173) it was shown that, by treatment with iodine in alcohol, long-chain monoepoxides could be smoothly converted to alkoxyhydroxy compounds whose TBDMS derivatives had excellent chromatographic and mass spectral properties. Reaction of long-chain epoxides with iodine in aqueous tetrahydrofuran gave vicinal diols which were readily converted to mono-TBDMS ethers but di-TBDMS derivatives were difficult to prepare. Iodine-catalyzed alcoholysis and hydrolysis of long-chain epoxides has the advantage of enabling such transformations to be carried out in the presence of other functions which are sensitive to the more usual acid catalysis. The present paper will describe the application of the iodine-catalyzed solvolysis reaction to a wider range of long-chain epoxides and the use of TBDMS derivatives in their analysis. The long-chain epoxides in this study are derived from polyunsaturated non-hydroxylated fatty acids and 3-hydroxy-2-branched (mycolic) acids of bacterial origin. The properties of TBDMS ethers of saturated 2- and 3-hydroxy esters will also be reported.

450

STUDIES ON THE INHIBITION OF LIPID PEROXIDATION IN RAT LIVER MICROSOMES. T.F. Slater and R.L. Wilson, Brunel University, Uxbridge, Middx, UK and J.Packer, Auckland University, N.Z.

Lipid peroxidation can be produced in various ways in microsomal membranes obtained from mammalian tissues: enzymically via NADPH-linked reductions (with or without added iron-chelates) or through the metabolism of numerous hepatotoxic agents (such as CCl_4), and non-enzymically (e.g., by radiation or by the addition of ascorbate). Different free radical initiating species are involved in these various processes, and the propagation stages may also be diverse. The processes of initiation and propagation can be attenuated by free radical scavengers, and fast-reaction kinetic studies on these reactions that inhibit lipid peroxidation have been made. In particular, the complex effects of phenothiazine, phenolic and metal chelating inhibitors will be outlined and attempts that have been made to discriminate between reactions involving $OH^{\cdot}$, $^1O_2$, $O_2^{-\cdot}$ and $CCl_3^{\cdot}$ will be presented. Data obtained by pulse radiolysis, and by ESR techniques will be described. The importance of inhibiting and controlling lipid peroxidation in vivo will be discussed in relation to cell injury and to prostaglandin synthesis.

451

ACETOLYSIS OF GLYCOPHOSPHOLIPIDS. N. Shaw, Microbiological Chemistry Research Laboratory, The University of Newcastle upon Tyne, Newcastle upon Tyne, England NE1 7RU.

Glycerophospho- and phosphatidylglycobiosyldiacylglycerols are known to be present in many bacteria and are structurally related to the ubiquitous diacylglycobiosylglycerols. A major problem in the structure determination is the point of location of the phosphodiester linkage to the sugar moiety of the glycolipid. Thus far it has not been possible to demonstrate directly the sugar-phosphate linkage in these glycophospholipids because the stereochemistry of the phosphodiester bonds dictates that on alkaline hydrolysis glycerol phosphates are the only monophosphates produced. A study of the products of acetolysis, a method previously used for the isolation of diacylglycerol acetates from phospholipids, has enabled this structural feature to be examined. Under suitable conditions acetolysis of phosphatidylglucobiosyldiacylglycerol isolated from *Streptococcus faecalis* gave a reasonable yield of a glucobiosylglycerol phosphate which on acid hydrolysis gave glucose and glucosylglycerol phosphate. Dephosphorylation of the latter gave glucosylglycerol and acid hydrolysis gave glucose 6-phosphate and glycerol. The isolation of glucose 6-phosphate demonstrates directly the presence of a sugar-phosphate linkage in the glycophospholipid and the degradative sequence shows that the phosphodiester residue is linked to the internal glucose moiety of the glucobiosylglycerol, thus confirming the structure originally proposed. In contrast, application of this method to the glycerophosphoglucobiosyldiacylglycerol from *Acholeplasma laidlawii* has shown that the glycerophosphate moiety is linked to the terminal glucose of the glycolipid as acid hydrolysis of the glucobiosylglycerol phosphate produced on acetolysis gave mainly glucose 6-phosphate and glucosylglycerol. The method is applicable to other glycophospholipids of this type.

452

APOLAR INTERACTIONS IN THE BIOSYNTHESIS OF LIPIDS: I. ARRANGEMENT OF ACYL CHAINS IN EUTECTICS OF FATTY ACIDS. Damascene Rebello and Kalpana J. Modi, University of Bombay, Department of Chemical Technology, Matunga, Bombay 400 019 India.

Mixtures of fatty acids were studied for their melting and solidification characteristics. Binary mixtures of (1) two saturated fatty acids, ranging from octanoic through behenic and any one unsaturated fatty acid, *viz.* oleic, linoleic, erucic or elaidic acid, exhibit eutectics at 24 clearly defined compositions, all containing less than 50 mol % of the higher melting component, with one exception at 66.7 mol %. Unsaturated fatty acids simulate short chain fatty acids in these eutectics. Mixtures of three fatty acids of the type C_n, C_{n+2}, C_{n+4} exhibit eutectics exactly in the molar proportion of 6:2:1, respectively, and the melting point of the eutectic in each case is lowered to that of the pure fatty acid, C_{n-2}. Mixtures containing two saturated fatty acids, C_n, C_{n+2} and oleic acid give eutectics containing the same ratio of saturated fatty acids (2:1 respectively) with predictable, varying amounts of oleic acid. Suggestions, based on the assumption of uniform apolar interactions between acyl components in eutectics, are offered for their uniform distribution in the crystal lattice of the eutectic in each system. Similar arrangements of components in mixed fatty acid pools are probably involved in the *de novo* biosynthesis of phosphatidic acid, as discussed in the next paper.

453

APOLAR INTERACTIONS IN THE BIOSYNTHESIS OF LIPIDS: II. A NEW MECHANISM OF *DE NOVO* BIOSYNTHESIS OF LIPIDS FROM POOLS OF PREARRANGED FATTY ACIDS. Damascene Rebello, Department of Chemical Technology, University of Bombay, Matunga, Bombay 400 019 India.

Mechanisms proposed for the *de novo* biosynthesis of phosphatidic acid *in vivo*, are not adequate enough to describe the asymmetric distribution of saturated and unsaturated fatty acids in these molecules. A new mechanism is proposed to describe the manner of utilization of fatty acids, prearranged in mixed fatty acid pools, by acyl transferases, leading to their asymmetric distribution in different biosynthesized phosphatidic acids. In the previous paper (452) suggestions were offered for the uniform distribution of components in eutectics of fatty acids based on uniform apolar interactions between acyl chains. In all probability, a similar

distribution occurs in mixed fatty acid pools, held in liquid crystalline state, in many biological systems, resulting in selective pairing of non-identical fatty acids, which could be predicted from a study of the eutectics. Phosphatidic acid is formed by sequential transfer of the more rapidly activated paired component as its CoA derivative, to the 1-position of *sn*-glycerol-3-phosphate, in the more common 1-acyl-*sn*-glycerol-3-phosphate pathway, followed by esterification of its paired partner in the pool to the 2-position, leading to asymmetric placement of saturated and unsaturated fatty acids in the resultant phosphatidic acid. In the 2-acyl-*sn*-glycerol-3-phosphate pathway, this placement will be reversed, as is observed in certain myobacterial. Pools of 1,2-diacyl-*sn*-glycerols, formed by hydrolysis of phosphatidic acids with phosphatidate phosphohydrolase, will be esterified to "free" activated fatty acids, giving triacyl-*sn*-glycerols. Fatty acids exhibiting weak apolar interactions, such as acetic, butyric, caprioc and atypical fatty acids; and those exhibiting strong apolar interactions, such as arachidic, behenic and lignoceric acids, which will not be held in liquid crystalline state in the "pool", will not participate in the *de novo* biosynthesis. These will however be easily esterified as "free" CoA derivatives to the 3-position of 1,2-diacyl-*sn*-glycerols, as is observed in natural biological systems.

453A

STUDIES ON THE CONTENT AND HYDROGENATION CONDITION OF SQUALENE FROM THE LIVER OIL OF DEEP SEA SHARK. Ching-Shyong Wu, National Taiwan College of Marine Science and Technology, Keelung, Taiwan, Republic of China.

The quality of liver oil of deep sea sharks of *Centrophorus,* caught in the water near Taiwan, were investigated. The results showed that the weight of the liver averaged about 25.3% of the body weight. The oil content of liver is 80.5 to 95.3%, and the hydrocarbon content in the liver oil is 61.7 to 94.0%. The hydrocarbon content varied with individual fish; however, it was independent of the time of catch and species difference. It is shown by gas chromatography that the hydrocarbon in the liver oil is mostly squalene. The relation between the hydrocarbon content and refractive index may be expressed by the following equation: $X = -4782.9839 + 3261.8653Y$, where X is the hydrocarbon content (%) in the liver oil, and Y is the refractive index (n_D^{20}) of the liver oil. Gas chromatography, using a 5% SE-30 column, iodine value, and refractive index were used to determine the squalane content during hydrogenation of squalene. The results showed that, using Nysel DM-3 as a catalyst, hydrogenation was not completed after 6 hours at 150 C and 60 psig of H_2 pressure, whereas at 200 C, the hydrogenation was completed within 3 hours.

453B

STILLINGIA OIL—A GOOD SUBSTITUTE FOR TUNG OIL. Kan Ching-hao, Liao Mei-teh, and Hu Ping-huang, Fukien Normal University, Foochow, China.

Stillingia oil, extracted (by pressing or solvent extraction) from the fruit of Stillingia Sebifera, is a good substitute for tung oil. This tree, historically used for soap and candles, is widely distributed in south and west China. Various reports on the properties of this oil have been presented, but none of them emphasized its constitution and the relation of its constitution to its other properties. From the inspection of its mixed glycerides and derivatives with spectroscopical analysis, a special triene acid was found; like eleostaric acid in tung oil, it played an important role in the drying property. The present authors tried to use this special oil. Its alkyd resins (prepared from this oil and mannitol) yielded good insulation material (grade B-F).

453 C and 453 D appear after Abstract 500.

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DETERMINATION OF FATTY ACIDS IN THE WEATHERED STONE OF SOME ITALIAN MONUMENTS. B. Sergio Curri and Andrea Paleni, Center of Molecular Biology, Via G. Cagliero, 10-20125 Milan, Italy.

In previous observations the presence of glycerides, phospholipids, sterols and other lipids have been ascertained in chloroform-methyl alcohol extracts from the flora pushing on marble columns of the Torcello's church Santa Fosca, from Etruscan and Greek ancient pottery and from the surface of a Carrara marble statue of the 18th century. To explain these findings, three hypothesis have been made: (a) surfaces pollution of environmental origin, (b) absorption into the stone pores and interstices of water-lipid emulsions coming from the metabolic products of soil microorganisms and (c) penetration inside the weathered stone of bacteria, microfungi and yeasts and progressive increase of the lipids and other metabolic products of these microorganisms, associated with their growth. In this case, the existence of relationships between the metabolic patterns of the stone microorganisms and the chemical characteristics of the lipids can be postulated. Extracts according to Folch have been made of samples of stone taken from the internal air of the Cathedral of Trani (A) and from the portal of the "Palazzo dei Principi" of Correggio (B) which suffering a rapid process of decay. The microbiological analysis of the stone show the presence of a microbic and mycetic aggression of the two monuments, but by different strains of microorganisms. Using TLC methods glycerides, phospholipids, hydrocarbons, sterols, linear alcohols and terpens have been identified. The GLC determination of fatty acids of A and B shows great differences of their percentual distribution: C_{16} and C_{18} was more than 100% higher in B than in A and inversely C_{12}, C_{16}, C_{18} and C_{20} show lower quantities in B. Our findings suggest that the hypothesis (c) is the most correct and emphasize the importance of the lipid analysis to ascertain the nature of the stone decay and the entity of the biological attack.

454A

NOTES ON THE EVOLUTION OF OIL PAINTING. Meryl Johnson, M. Grumbacher, Inc., 460 West 34th St., New York, NY 10001.

The author has identified the binding media in paintings of the Flemish and Italian Renaissance, the 16 to 19th centuries, and the modern period. Total acceptance of oil painting happened more slowly and conservatively than has been thought. Drying and yellowing problems had to be solved, along with the problem of using pigments which were dubiously adaptable to oil. The development of oil painting was encouraged by the notion that other masters had secrets for its use which could be imitated; for example, the Italians copied the Flemish, the Flemish copied the Italians, and, although both were using some oil, neither had fully mastered its use. Finally, the Royal Academies developed methods for repeating effects found in Renaissance paintings. The use of oil painting was not fully developed into the reliable working methods we have today until after the introduction of colors in tubes in the mid-19th century. This allowed manufacturers to standardize grinding methods and to develop a full palette of oil-compatible colors.

454B

LINSEED OIL AND RELATED MATERIALS: AN ANNOTATED BIBLIOGRAPHY OF MONOGRAPHS. N.S. Baer and R. Rushfield, Conservation Center, Institute of Fine Arts, New York University, New York, NY 10021; N. Indictor, Department of Chemistry, Brooklyn College, CUNY, Brooklyn, NY 11210.

This bibliography supplements previous annotated bibliographies by Baer and Indictor (*Art and Archaeology Technical Abstracts, IX* No. 1 Part I. From Antiquity to 1940; *IX* No. 2 Part II. From 1941 to 1960; *X* No. 1 Part III. From 1961-1972; *Lipids and Works of Art; Proceedings of the 13th I.S.F. Congress, 1976.* From 1973-1975; *Proceedings of the 14th I.S.F. Congress, 1979.* From 1976-1977.) Works dealing with the history, manufacture, chemistry, and use of drying oils are included, with particular reference to rare materials in private collections.

454C

THERMOGRAVIMETRY OF LINSEED OIL IN THE PRESENCE OF IRON-BASED, COPPER-BASED, AND CARBON BLACK PIGMENTS. N. Indictor, Department of Chemistry, Brooklyn College, CUNY, Brooklyn, NY 11210; C.J. Shahani and N.S. Baer, Conservation Center, Institute of Fine Arts, New York University, New York, NY 10021; M.J.D. Low and J. Chan, Department of Chemistry, New York University, New York, NY 10003.

Following previous reports describing thermogravimetric changes of linseed oil in the presence of metal acetylacetonates in the temperature range of 60 to 100 C, thermogravimetric changes have been observed on linseed oil samples containing iron-based, copper-based, and carbon black pigments. The results suggest that the pigments themselves have no measurable effect on the thermogravimetric drying rates of linseed oil under the experimental conditions employed. Inhibiton periods, rate of increase in weight, maximum weight achieved, and subsequent weight decrease are reported.

454D

CRITICAL EVALUATION OF PUBLISHED DATA ON THE COMPOSITION OF ANCIENT MATERIALS. SOME QUESTIONS OF RESEARCH AND PRACTICE. Stefan C. Arteni, American Romanian Academy, 34-41, 77th St., 127, Jackson Heights, New York, NY 11372.

The major failing with comments on the examination of paintings has been an unfamiliarity with the basic techniques of oil painting. Mistranslation of early MSS. can confuse the issue, and recent scientific examinations must be interpreted with caution. This article is the conclusion to the technical reports included in the I.S.F. papers. It deals with the author's investigation into various recipes for oil painting, including a review of materials and techniques and going into detail over treatment of ingredients. Differences among the treatments will be emphasized and data will be presented. The listing covers methods of painting and formulae from medieval times to the 20th century. Application techniques for the paint film are discussed. Topics discussed include the rela-

tionship between materials and techniques and the survival of the painting. Objectives are to record the principal methods, to study the properties of pigments and vehicles and to examine possibilities for utilizing these properties. The constituents of the paint layer, alterations, and the effect of mixing pigments are discussed, indicating a very elaborate technique. Raw materials are briefly discussed for those who want to control the quality of their materials by making their own.

454E

DRAWING MATERIALS, A PRELIMINARY STUDY. RESTORATION PROBLEMS OF DRAWINGS EXECUTED IN MIXED TECHNIQUES. Stefan C. Arteni, American Romanian Academy, 34-41, 77th St., 127, Jackson Heights, New York, NY 11372; Myriam Sanchez-Posada de Arteni, Schomburg Center for Research in Black Culture, The New York Public Library, New York, NY.

The materials used in drawings are discussed. The article is divided into chronological, historical segments beginning with the Old Masters's techniques and tools: metalpoint drawing, chiaroscuro drawing, and pen drawing; inks (black carbon, iron gall, bistre, sepia and colored inks and washes); chalks, pastels and crayons; and charcoal (including oiled charcoal) and graphite. The authors aim to supply a critical and historical study of drawing techniques. Over the last hundred years, water-based and oil-based paints have been extensively used as drawing media, either alone or in association with traditional materials. Conservation problems posed by these techniques are identified and practical answers discussed. The article is intended as a preliminary survey, to be further developed into a comprehensive study of drawings and their conservation.

454F

TECHNOLOGICAL INNOVATION AND MANUFACTURE OF OIL PAINTING MATERIALS: EVOLUTION FROM HANDCRAFT TO MASS PRODUCTION. Stefan C. Arteni, American Romanian Academy, 34-41, 77th St., 127, Jackson Heights, New York, NY 11372.

The composition and general properties of vehicles, varnishes, and so on are discussed, as well as paint formulation and manufacture. The manufacture of ready-made art materials flourished during the 17 to 18th centuries, whereas it did not thrive until the middle of the 19th century. The earliest records are reported. The author also discusses changes in painting which began in the 19th century, i.e., inferior, factory-made colors and canvases. The special problems posed by 20th century art are surveyed. Commercial materials are considered critically and various drawbacks described. Various unsatisfactory techniques and materials are mentioned. A broad survey of literature is presented (historical information from old MSS.) concerning the studio manufacture of oil colors, oil purification and varnish making. Convenient methods of making up oil colors, varnishes, and clarified oils are given. The notes and references contain the basic formulae used by the author in making his own materials. The small amount and value of materials involved require some practical and innovative application of these techniques. Difficulty has been experienced in finding raw materials. Our own approach has been to demonstrate that the formulations contained in old textbooks are consistent with sound practice. Whether a manufacturer accepts this responsibility is sometimes a question.

454 G

1968 START RESEARCH OF LIPIDS ON WORKS OF ART. Andrea Paleni, Societa Italiana Ricerche Agricole & Industriali, Casella Postale 104, 42015 Correggio (Reggio E.), Italy.

The public statement by G. Degelius, J.A. Hedvall and others that monocellular colonies were one of the causes for outdoor sculpture architecture and fresco deterioration goes back to the first years of our century. In 1968, when we started to deal with these problems in Venice, we found an environment which was unprepared to consider the idea of the existence of such a biological aggression to destroy architectural structures in spite of issue of J. Pochon, J. Jaton, W.E. Krubein, S.B. Curri, C. Jeason, and others. The publication of literature on the subject indicated our work would be relatively simple and our recommendations to disinfest the works of art which had been attacked would be accepted. Confronted with a stubborn disbelief, the only thing we could do was acquire experimental data on stone, including stone where biological aggression was not at all evident. We used thin layer chromatography on extracts with chloroform and methyl alcohol (3:1) from surfaces which were presumed infested. The method of extraction from surface of works of art on site was accepted as not damaging by the authorities. This analysis of crust present on the surface of marble columns of the Santa Fosca arcade at Torcello by TLC, GC, and MS confirmed once more the biogenic origin of these lipids and excludes the presence of incombustibles from hydrocarbons and smoke coming from factories of Marghera. In order to obtain more documentary evidence for biological aggression on the stone we used biological research which we will not go into here.

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RICINOLEIC ACID IN *PHYLLANTHUS NIRURI* (EUPHORBIACEAE) SEED OIL. Moghis U. Ahmad, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843, S.K. Husain and S.M. Osman, Aligarh Muslim University, India.

The fatty acids of *Phyllanthus* species were believed to be mixtures of linolenic, linoleic, oleic, and saturated acids. Linolenic acid represents about 35% of the total fatty acid mixture and thus these oils are classified as "linolenic-rich" type of oils. In addition, the Auphorbiaceae family was somewhat exceptional in possessing a few species which elaborated unusual acids in the seed fats; one of the most important ones is ricinoleic acid in the *Ricinus* species. In our search for the newer oils containing acids of novel structure, it was found that *Phyllanthus niruri* seed oil contains 1.2% ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid, previously unknown in the genus *Phyllanthus*. Structural details were elucidated through chromatographic techniques, IR, NMR, as well as chemical methods. Other major components of this oil are linoleic acid (21%) and linolenic acid (51.4%).

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POSITIONAL ISOMERS OF HEXADECENOATES ISOLATED FROM LIVER AND HEPATOMA LIPIDS. Randall Wood and Theresa Lee, Texas A&M University, Department of Biochemistry and Biophysics, College Station, TX 77843.

Cis-hexadecenoates were isolated from normal rat liver and hepatoma 7288CTC neutral lipids and phospholipids using argentation thin-layer chromatography and preparative gas-liquid chromatography (GLC). The hexadecenoates were analyzed for positional isomers by ozonolysis and capillary GLC. Normal liver hexadecenoates consisted of predominantly palmitoleate, the Δ^9 isomer, with small amounts of the Δ^6, Δ^7, and Δ^{11} isomers making up the balance. In addition to the hexadecenoate positional isomers found in normal liver lipids, hepatoma neutral and polar lipids contained relatively high percentages of Δ^{12} and Δ^{14} hexadecenoates. The occurrence of these unusual positional isomers in the hepatoma lipids was demonstrated by both analytical techniques. Examination of the possible origin of these unusual isomers suggests that they may result from an error in lipid metabolism in the hepatoma. (This work was supported by Public Health Service Research Grant No. CA-20136 from the National Cancer Institute.)

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STEREOSPECIFICITY OF DIFFERENT LIPASES. B. Åkesson, Department of Physiological Chemistry, P.O. Box 750, S-220, 07 Lund 7, Sweden; S. Gronowitz, B. Herslöf and P. Michelsen, University of Lund and T. Olivecrona, Section of Physiological Chemistry, Univ of Umeå.

By comparing the lipolysis of enantiomers of various acylglycerols, detailed information on the mechanism of lipase action can be obtained. The use of pairs of enantiomers has the advantage that no properties except the chirality of the compounds can explain a preferential lipolysis of one enantiomer. One approach in these studies involved the use of differently labeled enantiomeric alkyldiacylglycerols. Racemic mixtures of 1,2-di[^{3}H] oleolyl-3-tetradecyl-*sn*-glycerol and 1-tetradecyl-2,3-di[^{14}C] oleoyl-*sn*-glycerol were incubated with lipase from pancreatic juice, milk (bile-salt-stimulated lipase), Rhizopus arrhizus or Pseudomonas fluorescens. The first three lipases did not exhibit stereospecificity with this substrate since the ratio $^{14}C/^3H$ in the products was the same as that in the substrate. Another pattern was observed for P. fluorescens lipase. The total hydrolysis of both alkyldiacylglycerol isomers was the same but the relative amounts of lipolysis products differed. ^{3}H accumulated as free fatty acid and ^{14}C dominated over ^{3}H in alkylmonacylglycerol. This indicates that of the intermediate products, 2-oleoyl-3-tetradecyl-*sn*-glycerol was hydrolyzed faster than 1-tetradecyl-2-oleoyl-*sn*-glycerol. This stereospecificity has been confirmed using different alkylmonoacylglycerols as substrates. The data indicate that lipase from P. fluorescens has a type of stereo-sepcificity not previously described. Further studies with stereochemically defined synthetic acylglycerols are needed to define the structural requirements of this stereospecificity.

458

HORMONAL REGULATION OF Δ5 DESATURATION OF FATTY ACIDS. Roldolfo R. Brenner, Irma N.T. de Gómez Dumm and María J.T. de Alaniz, Instituto de Fisiología, Fac. de Ciencias Médicas, U.N.L.P. Calle 60 y 120, 1900-La Plata, Argentina.

We have established that hormones regulate the Δ9 and Δ6 desaturation of fatty acids in animals. This line of research has been extended to study the effect of different hormones and dibutiryl cyclic AMP on the oxidative desaturation of eicosa-8,11,14-trienoic

acid to arachidonic acid in rat liver microsomes and HTC cultured cells. It was demonstrated that the administration of epinephrine to rats produces a significant decrease on liver Δ5 desaturation activity either after short or long terms of treatment. However, the addition of epinephrine to HTC cultured cells incubated with 1-^{14}C eicosa-8,11,14-trienoic acid produced no changes in arachidonic acid synthesis, but the incubation with dibutiryl cyclic AMP resulted in a significant decrease on Δ5 desaturation activity. Therefore, this result suggests that epinephrine receptors are absent in this kind of cells, and that the possible mechanism of epinephrine action on Δ5 desaturation is evoked through cyclic AMP increase. Glucocorticoids also decrease the microsomal activity of the Δ5 desaturation of eicosa-8,11,14-trienoic to arachidonic acid in the rat. However, although HTC cells respond to dexamethasone treatment increasing tyrosine amine transferase activity, they show no changes on Δ5 desaturation activity. Besides, the arachidonic acid synthesis decreased by the addition of dibutiryl cyclic AMP, is not potentiated by the presence of dexamethasone. The changes found on the Δ5 desaturation activity are compared to the results obtained with Δ6 and Δ9 desaturases.

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FURTHER STUDIES ON THE BIOCHEMICAL AND PHARMACOLOGICAL ANTAGONISM OF THE UNSATURATED AND SATURATED FATTY ACIDS IN SUPPORT OF REVICI'S DUALISM. Benedict B. O'Malley, Department of Chemistry- Room S-428, Jersey City State College- 2039 Kennedy Blvd., Jersey City, NJ 97305.

Tetrahymena pyriformis has been used as a biochemical tool since 1924. (Elliot in 1959 reviewed its important role in biochemistry, biology and pharmacology in a classic paper-"A Quarter Century Exploring Tetrahymena."). This investigator has published extensive papers on the biochemistry, pharmacology and physiology of this ciliate since 1949. Since joining oncologist Revici in 1958, his attention has been focused on a study of the fatty acids in a variety of cell systems. Kabara in 1978 and 1979 focused attention on the importance of the lipids in extensive reviews on the subject. O'Malley and Lurie reported in 1979 on the biochemistry and pharmacology of the oxyconjugated fatty acids on the growth and metabolism of Tetrahymena. (Am. Chem. Soc. Oct. 1979). A preliminary report was made on the antagonism between the oxyconjugated fatty acids and the saturated fatty acids in terms of growth and metabolism of Tetrahymena. This report presents evidence that indicates the increase and extension of viable forms of axenic Tetrahymena with acrylic, linoleic, linolenic, muconic, fumaric and citraconic acid-with YSI readouts indicating oxygen uptake. While saturated acids-lauric, palmitic, myristic acids depress growth and YSI records indicate no O_2 uptake. The results indicate further proof of the validity of Revici's Theory of Dualism, "Research in Physiology As Basis of Guided Chemotherapy with Special Application to Cancer." (Published by Van Nostrand-1961-Emmanuel Revici, M.D.

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LIPID BIOSYNTHESIS IN DEVELOPING SUNFLOWER *(HELIANTHUS ANNUUS)* SEEDS. Manuel Mancha and Juan Sanchez, Instituto de la Grasa, Av. P. Garcia Tejero, 4. Sevilla-12. Spain.

The capacity of lipid synthesis in developing sunflower *(H. annuus)* seeds from radioactive fatty acids has been examined. Lauric, palmitic, stearic and oleic acids were tested as precursors. In both intact tissue and a free cell extract, containing soluble and microsomal cell fractions, an active triglyceride synthetase activity was found. The analysis of acylthioesters synthesized from the four fatty acids showed that only lauric acid can be bound to acyl carrier protein, but all of them can be activated to ester of coenzyme A. Lauric acid was elongated to stearic acid. Oleic acid was desaturated to linoleic acid. Palmitic and stearic acids were not modified although incorporated, as the other acids, into different lipid classes, namely polar lipids, diglycerides and triglycerides. Positional analysis showed that, in general, the labeled fatty acids were preferently incorporated into the 1,3 positions of triglycerides and 1 position of diglycerides and polar lipids, oleic acid being the only exception.

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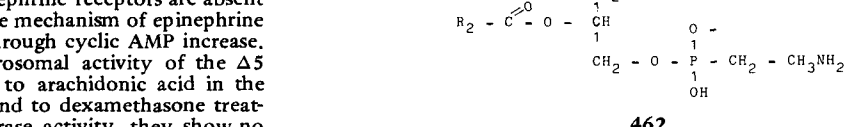
PRELIMINARY IDENTIFICATION OF A MONOVINYL-ETHER, MONOFATTY-ACYL PHOSPHOGLYCERIDE FROM *T. VIVAX* BY PAPER CHROMATOGRAPHY. Gregory E. Anekwe, Department of Biochemistry, College of Medicine, University of Lagos.

The salivarian trypanasome *T. Vivax* was separated from rat blood by the method of Lanham, utilizing phosphate-Saline glucose, pH 8. The sample was centrifuged at 1800 g for 20 min. to remove red cells. Lipid extraction from the blood-free sample was carried out with methanol/chloroform (2/1) v/v, followed by chloroform/methanol (1/1) v/v, and purification was performed by gel filtration, utilizing sephadex-G-25. The lipids were separated into neutral and polar lipid fractions by silicic acid column chromatography and the polar lipid fractions separated by thin-layer-chromatography. The phosphoglyceride fraction of the polar lipids was subjected to mild alkaline hydrolysis followed by mild acid hydrolysis according to Dawson. The product of the hydrolysis was subjected to paper chromatography, and the resultant spot identified by indicator reagents. The results suggested the presence of ethanolamine plasmalegen in *T. Vivax:*



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PHYSICO-CHEMICAL STUDIES ON THE SULFONIUM ANALOG OF LECITHIN IN DIATOMS. M. Kates and P.-A. Tremblay, Department of Biochemistry, University of Ottawa, Ottawa, Canada K1N 6N5.

Marine diatoms contain phosphatidyl sulfocholine (PSC) as a major membrane component which completely replaces the usual phosphatidyl choline (PC) in at least one species, *Nitzschia alba.* This finding raises questions concerning the structure-function relationship of the sulfonium analog as a membrane component compared to that of phosphatidyl choline. We have recently synthesized a homologous series of PSCs (di-14:0, di-16:0, di-18:0 and di-18:1) and have compared their physical properties with those of the corresponding PCs. All of the PSCs had higher (2–4 C) transition temperatures than the corresponding PCs, as measured by differential scanning calorimetry (DSC), ESR studies with 5-doxyl stearic acid as spin probe, calorimetry (DSC), ESR studies with 5-doxyl stearic acid as spin probe, and fluorescence polarization studies with diphenylhexatriene as probe. The sulfonium analogues thus appear to form more stable bilayers than the ammonium analogs, and this might be due to a stronger polar interaction of the sulfocholine headgroup compared to the choline headgroup.

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THE TRIGLYCERIDES OF SAPROPHYTIC MYCOBACTERIA. T.B. Kornelli, Moscow State University, Biological Department, Moscow W-234, USSR; and B.V. Rozynov, M.M. Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow.

The triglycerides of mycobacteria and artrobacteria grown on media with hexadecane or glucose were studied by means of mass spectrometry. It was showed that the triglycerides represent the mixtures of homologues in all cases. The triglycerides from *Arthrobacter ceroformans* formed with using hexadecane consist of 90% of tripalmitate and 10% of triglyceride generated by two residua of palmitic and one residuum of palmetoleic acids. In cells of mycobacteria grown on hexadecane-containing medium prevail over the mixed triglycerides containing one unsaturated acylic group. The acylic groups represent the residua $C_{14:0}$-, $C_{14:1}$-, $C_{16:0}$-, $C_{16:1}$-, $C_{17:1}$- acids. It determined the location of the different acylic residua in the mixed triglycerides and quantitative correlations between homologous compound in mixtures. The triglyceride fraction from mycobacteria grown on glucose-containing medium are considerably more complex mixture: the number of the main components grow up but the number of the minor ones represent a full set of homologues with molecular weights from 776 to 888.

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THE ROLE OF LIPOXYGENASE IN THE DEVELOPMENT OF VEGETABLE FLAVORS. S.J. Kazeniac and E.J. Stone, Campbell Institute for Research and Technology, Campbell Place, Camden, NJ 08101.

Lipoxygenases react with linoleic and linolenic acids with a high degree of specificity to initiate reactions that lead to important flavor compounds in fruits and vegetables. Disruption of cellular tissue activates the enzymes and starts the various reactions that yield the broad spectrum of volatile compounds characteristic of the particular food. Biogenetic pathways that lead to the development of lipoxygenase-mediated volatile compounds will be briefly reviewed. Data will be presented to show how these flavor compounds originate in tomatoes, green beans and mushrooms. Relations of preparative or processing methods, maturity/ripeness and physical condition to the flavor quality of these vegetables will also be discussed.

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INVESTIGATIONS ON OXIDATIVE STABILITY AND ITS KINETIC CONTROL IN SOME FATTY FISH LIPIDS. J.K. Kaitaranta and P.J. Ke, Department Fisheries & Oceans Canada, Maritimes Fisheries Management, 1971 Lower Water Street, P.O. Box 550, Halifax, Nova Scotia, Canada B3J 2S7.

A micro-method based on the weight gain and the formation of secondary oxidation products has been applied for the estimation of oxidative stability of the lipids from tuna, herring and redfish.

By using the sample from mackerel as the reference, the relative oxidative stabilities have been compared. Kinetic control of lipid oxidation of different fish lipids has been investigated by adding various biochemicals, food additives, natural spices as well as BHA and TBHQ. Differential oxidative stabilities of the component lipids have also been studied and discussed by the application of quantitative TLC-FID techniques.

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THE EFFECT OF DOCKAGE ON THE OXIDATIVE STABILITY OF RAPESEED OIL. N.A. Michael Eskin, Department of Foods and Nutrition, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada; F. Ismail, University of Cairo, Egypt; M. Vaisey-Genser, University of Manitoba.

The effect of dockage on rapeseed oil quality was investigated as a result of concern that seed contaminants may interfere with the processing of rapeseed. Two samples of dockage expeller and extractor oils and a rapeseed expeller and extractor oil were degummed, bleached and deodorized. The refined oils from the expeller and extractor oils were mixed in the ratio of 2:1 to simulate the oil obtained by normal processing of rapeseed. The combined dockage oils were added to the rapeseed oil at different levels corresponding to 0, 0.5, 1.0, 2.0 and 4.0%. These samples were subjected to accelerated storage at 65 C for 9 days. Assessment of the quality of the oil was monitored by measuring peroxide value, hydroperoxide value, TBA, free fatty acids and sensory analysis. The peroxide and hydroperoxide values were significantly ($P<0.05$) different in oil samples containing 1% and higher levels of dockage oil while TBA showed significant ($P<0.05$) differences above the 2% level of added dockage. The free fatty acid levels increased significantly ($P<0.05$) at 0.5% of dockage oil and above. No significant differences were evident from the sensory analysis of the oils.

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EFFECT OF PEROXY RADICAL SCAVANGERS ON FLUORESCENT LIGHT INDUCED OXIDATION IN SOME EDIBLE OILS. A. Asghar, Meat Sci. Lab. 112, Oklahoma State University, Stillwater, IK 74074; Pervaiz Akhtar, University of Agriculture, Faisalbad; and Adbus-Salam Sheikh, PCSIR Laboratories, Lahore.

Antioxidative effect of α-tocopherol, butylated hydroxy anisole and butylated hydroxy toluene at various concentrations (0.01–0.08%) was determined during light-catalyzed oxidation of almond, corn, cottonseed and rapeseed oils. Oxidation rates were measured in terms of peroxide values of the oils at different intervals of time. The infrared and ultraviolet spectra of the oils were also taken. It was observed that α-tocopherol was not an effective antioxidant. Butylated hydroxy anisole (BHA) showed antioxidative activity. However, butylated hydroxy toluene (BHT) was found more effective as an inhibitor of the photooxidation of these oils. The infrared spectra taken were typical of a common fixed oil and did not show any characteristic change such as geometrical isomerization. However, ultraviolet spectra did indicate some changes in certain oils as a result of photooxidation.

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APPLICATION OF THE FLUORESCENT LIGHT TEST AND SOME OTHER METHODS FOR DETERMINATION OF THE STABILITY OF DIFFERENT EDIBLE OILS. Biserka Matijasevic, Jovan Turkulov, Djerdj Karlović and Vjera Radenković, Faculty of Technology, Novi Sad, Oil and Fat Department, Veljka Vlahovica 2, Yugoslavia.

For determination of the stability of different kinds of edible oils (sunflower, corn, and soybean), various methods have been applied: fluorescent light test, AOM-test, oven-method, and stability at room temperature. Before testing stability, the fatty acid composition and tocopherol content and the quality characteristics (peroxide value, anisidine value, content of diene and triene) were determined. Special attention has been given to examining the stability by the fluorescent light test. This test seems to be very interesting in determining the oil stability because the action of the light is the main factor for the deterioration of the oil while it's in the supermarket in the clear glass or plastic bottles (in Yugoslavia, oil is not packed in brown glass). In the apparatus (made in our laboratory), oil in one-liter original glass or plastic bottles or in 50 ml jars were exposed to the light of four 40 Watt fluorescent cool-white tubes of 1 m. Oxidation rate of the samples was followed by measuring peroxide values and organoleptic changes. For the organoleptic evaluation, three panelists judged the odor and the flavor on a 10-point scale. The results show that, under conditions of this test, corn oil has the best stability. Sunflower oil has 2-1/2 times better stability than soybean oil. Some correlation has been established between the stability obtained by all applied method and the AOM-test having in mind that this is a standardized method for fat and oil stability evaluation.

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ANTIOXIDANT FROM FERMENTED BLACK SOYBEAN. T.Y. Liu and H.J. Chen, Food Industry Research & Development Institute, P.O. Box 246, Hsinchu, Taiwan, R.O.C.

Toshi, a product of black soybean (*Glycine max* L. Merril var. O'Tau) fermented by *Aspergillus soyae*, is widely used as seasoning in Chinese cuisine such as in the case of steaming fish or sparerib. The crude oil extracted from toshi was found unusually stable toward oxidation. When toshi oil was kept at 45 C under light for 30 days, its peroxide value was not significantly changed and the α-carotene contained in the oil was still not completely oxidized, whereas, the peroxide value of the crude oil from black soybean increased from 4 to 200 meq/kg under the same condition. Toshi oil was fractionated into six fractions by silicic acid column chromatography. One fraction with strong antioxidative activity was further investigated by means of TLC, IR and UV spectrophotometry. The strong antioxidative activity was attributed to 2,4,6,3^1,4^1-pentahydroxychalconol. Further studies indicated the active compound was derived from cyanidin-3-glucoside during fermentation. Cyanidin-3-glucoside isolated from seed coat of black soybean was hydrolyzed with hydrochloric acid. After adjusting pH to neutral, the hydrolyzed product displayed strong antioxidative activity. The IR and UV spectra of this compound were same as those found in toshi oil.

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POTENTIAL ANTIOXIDANTS FROM *PIPER BETLE* L. W.H. Chang and K.S. Wang, Graduate Inst. of Food Sci. & Technol., National Taiwan Univ., Taipei, Taiwan, R.O.C.

Piper betle L. is a kind of special spice grown in Southeast Asia; the green leaves and small flowers are widely consumed by Asians, almost exlusively as a component of "pin-lang", which is a chewing recreation food composed mainly of the fruits of *Areca catechu* L. The leaves, vines and flowers of the plant were separately air-dried and pulverized. The dry powder was first extracted with methanol, and then twice with n-hexane. The two extracts were separately subjected to vacuum concentration until solvent-free, and the residual liquid was then extracted with ethyl ether. The two ethereal extracts, after washing with water, were vacuum concentrated to give oily products, the yields of which were 1.5%, based on the weight of the dry powder, from the methanol extract and 0.5% from the n-hexane extract. The oily products, or BHT, were added at 0.02% and 0.06% concentrations to fresh lard in petri dishes, and the mixtures were incubated in an air-circulated oven at 98 C. The POV of each lard sample was measured during the incubation, and the time length elapsed to reach a POV over 20 was compared. The results were: (1) the methanol and the n-hexane extracts of the 3 parts of the plant all showed potent antioxidant activity; (2) the methanol extracts obtained from the 3 parts all exhibited stronger (1.3–1.9 times) antioxidant activity over the respective n-hexane extracts; (3) the methanol extract of the vines showed the highest antioxidant activity, which was 1.2–2.2 times as that of BHT under the same experimental conditions. The methanol extract of the vines was found by silicic acid column chromatography, with a gradient mixture of ethyl ether and n-hexane as the elution solvent, to contain at least 2 major components with strong antioxidant activity.

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THE ROLE OF PROTEIN IN INTESTINAL FATTY ACID ABSORPTION. D. Sklan, Columbia University College of Physicians and Surgeons, 630 W. 168th St., BB 910, New York, NY 10032.

Fatty acid uptake in ligated intestinal segments in the chick from bile acid micelles was reduced in the presence of bovine serum albumin, casein or β-lactoglobulin, and a parallel inhibition of bile acid uptake observed. Proteolysis with pepsin or trypsin annuls this effect, which would appear to be due to binding of both fatty and bile acids by the proteins. Uptake of fatty acids complexed to the above proteins occurs in the intestine in the absence of bile acids, apparently by passive diffusion, at a rate about one third of that found from micellar solution. Lipophilic proteins thus have a dual action on intestinal uptake of fatty acids, depressing uptake by reducing micellar concentration, and in brush border where absorption occurs, more slowly, however, than from micelles. *In vivo* feeding experiments with increasing proportions of casein as the sole dietary protein revealed that when 45% casein was present in the diet, overall fatty acid absorption was depressed by some 13% compared to diets containing 30% or less casein. A similar trend was noted for bile acid excretion.

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PHOSPHOLIPIDS AND FATTY ACID CHANGES IN PERIPHERAL NERVE DURING WALLERIAN DEGENERATION. Jeffrey K. Yao, Rm 812 Guggenheim, Mayo Clinic, Rochester, MN 55901; V. Natarajan, Harald Schmid, The Hormel Institute, Austin, MN, and Peter J. Dyck, Mayo Clinic, Rochester, MN.

The phospholipid and fatty acid composition of phospholipids of the endoneurial portions of rat sciatic nerve were studied after unilateral crush injury, so that the net effect of varying combi-

nations of Wallerian degeneration and regeneration could be evaluated together and serially. The endoneurial total phospholipid and plasmalogen content (nmol/mg dry weight) decreased appreciably at 12 days after crush (50% and 25% of controls, respectively). By the time that nerve fiber regeneration had become well established (60–180 days), both phospholipid and plasmalogen content had returned to near normal (uncrushed) values. The polyunsaturated fatty acid of endoneurial phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were markedly altered when nerve underwent degeneration and regeneration. The proportions of 18:2 and of 20:4ω6 to total fatty acids were increased significantly ($P<0.001$) while proportion of 20:3ω6 was decreased proportionately. The ratio of 20:4ω6 to 20:3ω6 in PE and PC fractions increased and reached a maximum level (4–6 fold) at 12 and 30 days after crush injury respectively. On the other hand, the ratio of 22:4ω6 to 20:4ω6 decreased slightly (∿50%) for the same periods of time. At times after crush, when nerve fiber degeneration is superseded by fiber regeneration–at 60 and 180 days–the ratio of 20:4ω6 to 20:3ω6 still remained higher in regenerated nerve than in contralateral uncrushed nerve. Therefore, the fatty acid composition of endoneurial phospholipids appears to shift toward more unsaturated members of the ω6 series, particularly toward 20:4ω6, during nerve regeneration. The composition of alk-1-enyl moieties of endoneurial plasmalogens also changed markedly following crush injury. An increase of 16:0 with a decrease of 18:0 in the alk-1-enyl moieties were observed in nerve fiber degeneration, while an increase of 18:1 was found in regeneration.

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AORTIC GLYCOSAMINOGLYCAN CHANGES IN RABBITS FED SEMIPURIFIED DIETS: EFFECTS OF SUCROSE AND LACTOSE. Janet L. De Hoff, University of Pennsylvania, 998 Somerdale Road, W., Somerdale, NJ 08083, and David Kritchevsky, Wistar Institute.

Plasma lipoprotein [LP] fractions may have differential effects on arterial wall connective tissue metabolism in the early phases of atherogenesis. This study was designed to evaluate the role of carbohydrate as an initiating factor for arterial wall glycosaminoglycan [GAG] changes. Male Dutch Belted rabbits were fed sucrose or lactose as 40%, w/w, of a semipurified, cholesterol free diet for 10 weeks. The effects of the two diets on aortic lipids, plasma lipids, LPs and aortic GAGs were compared. At the end of the feeding period there was no macroscopic evidence of atherosclerosis. The plasma lipid data (mg/dl ± S.E.M.) are summarized below.

	TC	PL	TG
Sucrose, n = 5	345 ± 94*	239 ± 68*	285 ± 163*
Lactose, n = 8	102 ± 22	76 ± 10	56 ± 7

TC = total cholesterol, PL = phospholipid and TG = triglyceride. *denotes a significant difference (P 0.05).

Lipoprotein analysis on pooled plasma from the groups showed that the sucrose fed animals had higher apo-B containing lipoproteins (VLDL, IDL and LDL) than the lactose fed animals. The ratio of apo-B containing lipoproteins to high density lipoprotein was 17.0 in the sucrose pool and 4.2 in the lactose pool. Changes in the aortae are summarized below.

	TC[a]	PL[b]	GAG[c]
Sucrose, n = 4[d]	4.93 ± 0.44*	16.26 ± 3.94*	17.2 ± 1.5*
Lactose, n = 8	3.25 ± 0.17	9.85 ± 0.53	13.1 ± 0.2

[a]mg lipid/g dry delipidated aorta [dda]; [b] lipid/g dda and [c]micromoles GAG monosaccharide/g dda. Micromoles total GAG monosaccharide [e]equals micromoles hexosamine plus micromoles hexuronic acid. [d]Four out of five sucrose fed animals developed significant hyperlipoproteinemia. Analysis of aortic GAG changes in hyperlipoproteinemia sucrose fed animals are compared to the aortae of lactose fed animals.

In the lactose group there were minimal elevations of plasma lipids and the concentration of aortic lipids and GAG were lowest. The sucrose diet resulted in the highest elevation of plasma lipids and there were correspondingly higher levels of lipids and GAGs in these aortae. Preliminarily the aortic changes seem to correlate with the elevation of apo-B containing lipoproteins. The concomitant rise in aortic GAGs and lipids with elevations of plasma cholesterol seems to suggest that hyperlipoproteinemia is a necessary factor for aortic changes in diets free of cholesterol. Current studies are in progress to examine progressions of these changes in longer feeding experiments and with diets containing small (0.1%) levels of cholesterol. These studies will entail analysis of lipoprotein apoproteins and individual GAG analysis.

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MONOGLYCERIDES AS FOOD FAT FOR THE SUPPLY OF ESSENTIAL FATTY ACIDS IN CONDITIONS OF IMPAIRED LYMPHATIC FAT TRANSPORT. A. Christophe and G. Verdonk, Laboratory for Gerontology, Dietetics and Nutrition Research of the State University of Gent, Pasteurlaan 2, B 9000 Gent, Belgium.

Monoglyceride loading results in the postprandial state in a much lower chylomicronemia, triglyceridemia and shift in fatty acid pattern of serum triglycerides and non-esterified fatty acids toward that of the fat fed than natural fat loading, although fecal fatty acid excretion is comparable. After substitution of monoglycerides for natural fats in the diet of patients with chylous ascites of different origin, chylomicron and triglyceride concentration in ascites fluid was reduced to very low levels. Partial glycerides could not be detected in ascites fluid nor in serum. These results indicate that a considerable amount of fatty acids fed as monoglycerides (mainly α-isomers) are transported by the portal vein. If so, monoglycerides rich in essential fatty acids (EFA) would supply EFA in an effective way in conditions of impaired lymphatic fat transport. This was proven in an 18-year-old patient with exsudative enteropathy due to congenital lymphangiectasy who had been fed during several years a diet poor in fat and who showed biochemical signs of EFA deficiency (high triene-tetraene ratio; extremely low levels of linoleic acid in serum phospholipids and triglycerides). One week after institution of a diet with 30 g monoglycerides with the fatty acid pattern of safflower seed oil, there was an increase in linoleic acid and arachidonic acid content in serum triglycerides, phospholipids and cholesteryl esters to almost "normal" values and a reduction of the trienetetraene ratio. Exact time course of the changes in the concentration and composition of the major serum lipid classes will be presented. The results indicate that EFA can be effectively fed as monoglycerides in conditions of impaired lymphatic fat transport.

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SPECIFIC CHANGES OF BILE ACID METABOLISM IN SPONTANEOUSLY DIABETIC WISTAR RATS. A. Hassan, Lipid Research Center, Cincinnati General Hospital, K-4 Pavilion, 234 Goodman St., Cincinnati, OH 45267; M.T.R. Subbiah, University of Cincinnati Medical Center; and P. Thiebert, Health and Welfare Canada, Ottawa.

While alterations in plasma cholesterol levels have been noted in patients with diabetes mellitus and in alloxan induced diabetic animals, very little is known regarding bile acid metabolism in this disease process. Bile acid metabolism has been investigated in a newly described animal model depicting juvenile human diabetes (spontaneously diabetic Wistar (BB) rat) and compared to normoglycemic control from the Wistar strain. Diabetic animals used were on insulin treatment except for the last 24 hours. The plasma glucose levels (mg%) of diabetic rat (D) was significantly higher than control rats (C) (150 ± 35 in C vs 340 ± 32 in D). The total bile acid pool (mg/100 gm) in D was significantly ($P<0.05$) higher when compared to N (9.0 ± 0.8 in C vs 14.9 ± 1.7 in D). The pool of cholic acid was significantly ($P<0.05$) increased while that of chenodeoxycholic acid was significantly ($P<0.05$ decreased (cholic acid: 5.9 ± 0.45 in C vs 10.06 ± 1.2 in D; chenodeoxycholic acid: 0.90 ± 0.1 in C vs 0.57 ± 0.06 in D). This increased the cholic/chenodeoxycholic acid ratios from 6.6 ± 0.4 in controls to 19.3 ± 2.4 in diabetic rats. These studies have shown diverse alteration in the concentration of the two primary bile acids in the diabetic rat and suggests that insulin might play a role in the control of specific pathways of bile acid biogenesis.

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THE ROLE OF LIVER PEROXISOMAL β-OXIDATION IN THE METABOLISM OF VERY LONG-CHAIN MONOUNSATURATED FATTY ACIDS. M.S. Thomassen, C.E. Neat, H. Osmundsen, E.H. Christiansen, and K.R. Norum, Institute for Nutrition Research, University of Oslo, Blindern, Oslo 3, Norway.

The significance of the peroxisomal β-oxidation system in the total cellular oxidation of fatty acids is not fully understood. As yet it has only been manipulated by the use of drugs such as clofibrate. Very little has been done to investigate the effect of different diets on this system. When peroxisomes were isolated from livers of rats fed on a diet containing 30 kcal% of partially hydrogenated marine oil a 2,4 fold increase was found in the palmitoyl-CoA dependent NAD^+ reduction as compared to a control value for animals fed a diet containing 10 kcal% of soybean oil. A smaller (1,4 fold) increase was also observed when rats were fed on a diet containing 30 kcal% of soybean oil. This increase in peroxisomal β-oxidation closely correlates with changes in the rate of erucic acid oxidation in hepatocytes isolated from rats fed on the same three diets. These observations suggest that an increase in the peroxisomal β-oxidation capacity may be the main reason for the adaptation of very long-chain monounsaturated fatty acid metabolism seen in the liver after feeding rats diets containing such acids, i.e. partially hydrogenated marine oils and rapeseed oil.

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METABOLIC SIGNIFICANCE OF PEROXISOMAL β-OXIDATION' H. Osmundsen and C.E. Neat, Institute for Medical Biochemistry, University of Oslo, Box 1112, Blindern, Oslo 3, Norway.

The presence of peroxisomal β-oxidation in liver is now well established. Studies of peroxisomal β-oxidation have been undertaken to attempt to gain some insight into the metabolic role of this activity. β-Oxidation activity has been found to increase up to 450% in peroxisomes isolated from rats fed on high fat (15% by wt.) diets rich in long mono-unsaturated fatty acids (e.g. partially hydrogenated marine oil, rapeseed oil). Rats fed on soybean oil or ground nut oil diets gave marginal increases in peroxisomal β-oxidation. Peroxisomal oxidation of various fatty acids has been investigated. The rats of oxidation of trans-$C_{14:1}\omega 9$ and trans-$C_{16:1}\omega 9$ were 40–50% faster than those of the corresponding *cis*-isomers. With $C_{18:1}\omega 9$ the two isomers were oxidized at similar rates. With liver mitochondria, in contrast, the *trans*-isomers of $C_{16:1}\omega 9$ and $C_{18:1}\omega 9$ and were oxidized at rates which were about half those of the corresponding *cis*-isomers. With $C_{14:1}\omega 9$ the mitochondrial rates of oxidation were similar for the two isomers. Estimates of the total peroxisomal β-oxidative capacity in the rat liver suggest that this may represent 10–20% of the mitochondrial β-oxidative capacity, as regards oxidation of palmitate when peroxisomal β-oxidation has been induced by high fat diets. For erucic acid this value can be as high as 30%. Peroxisomal β-oxidation may be regulated by CoA in a manner which facilitates oxidation of long mono-unsaturated fatty acids (e.g. erucate) in preference to shorter fatty acids (e.g. palmitate). The above findings indicate that peroxisomal β-oxidation can be of particular significance as regards cellular oxidation of fatty acids which are not good substrates for mitochondrial β-oxidation.

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NUTRITIONAL STUDIES OF BI-CYCLIZED FATTY ACIDS IN THE RAT. A. Hase, Helsinki, University of Technology, Laboratory of Industrial Chemistry, 02150, Espoo 15, Finland, and Leena Pekkanen, Finnish State Alcohol Monopoly.

The unfavorable nutritional factors in tall oil fatty acids were studied using weanling male rats as test animals. A tall oil fatty acid fraction consisting mainly of two bicyclic pentyltetrahydroindanylbutanoic acid isomers was isolated using urea adduct formation, argentation countercurrent distribution and silica column chromatography. This fraction was tested in the diet of rats and compared to the original tall oil fatty acids and to a mixture of aromatized fatty acids isolated from tall oil fatty acids. Both the aromatized fatty acid fraction and the bicyclized fatty acids have an unfavorable effect on the growth of the test animals but cannot explain the total detrimental dietary effect of the tall oil fatty acids in rat diet.

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RESULTS FROM SUBSISTING ON AN ESKIMO DIET FOR 100 DAYS. H.M. Sinclair, International Institute of Human Nutrition, High Street, Sutton Courtenay, Oxon, OX14 4AW, England.

Eskimos on their traditional diet had the highest intake of fat in the world, but relatively very unsaturated; they had a very low incidence of non-infective "Western" diseases. For this reason I studied them in 1944 and was invited by Drs. Bang and Dyerberg to investigate long-lived Eskimos in Greenland in 1976. To follow various changes in body composition and function, I subsisted on only marine animal food (seal, fish and crustaceans) and water for 100 days. This diet is very low in the linoleic class of essential fatty acids and very high in the linolenic class, especially C20:5 and C22:6. It is also very high in cetoleic acid ($C22:1\omega 11$), in cholesterol and in retinol, but very low in ascorbic acid; it contains no carbohydrate or fiber. Linoleic acid decreased in body tissues (plasma lipids, erythrocyte membranes, platelets, adipose tissue, muscle) and was partially replaced by C20:5 and C22:6. Cetoleic acid appeared in adipose tissue and other lipids, and probably accounted for a very severe decrease in platelets (and altered morphology) and decrease in erythrocytes. But platelet aggregation increased quickly and to very high values. This can be explained by 3-series prostaglandins being formed instead of those of series 1 and 2. Despite the diet being very high in cholesterol, plasma cholesterol was low, VLDL being extremely low, LDL very low and HDL high. Plasma retinol became very high; ascorbic acid was zero in plasma and in white cells, but there were no clinical signs of scurvy; some spontaneous haemorrhages were attributed to the extreme "unstickiness" of platelets. The lipids of fish afford a very desirable dietary supplement to increase platelet disaggregation and so prevent thrombotic disorders such as ischaemic heart disease. But further work is required to study how small an amount will achieve this without toxicity of cetoleic acid.

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VERNONIA GALAMENSIS SEED OIL: A NEW SOURCE FOR EPOXY COATINGS. Kenneth D. Carlson, Wilma J. Schneider and Lambertus H. Princen, Northern Regional Research Center, AR/SEA, USDA, 1815 N. University St., Peoria, IL 61604.

A native African plant, *Vernonia galamensis*, is an excellent source of epoxy acid-containing triglyceride oil. The seed contains 40% oil, and the vernolic (12,13-epoxy-*cis*-9-octadecenoic) acid content of the oil falls in the range 72–78%. Processing conditions have been explored for cleaning, tempering, and flaking the seed; efficient extraction and recovery of the crude oil; and subsequent oil refining steps. As is characteristic of *Vernonia* species, rapid lipolytic activity in crushed *V. galamensis* seed leads to high free fatty acid levels in subsequently extracted oil if proper precautions are not taken in the processing steps. The film-forming properties of *V. galamensis* oil were evaluated by spreading the oil on steel panels, which were then baked for various times and temperatures with and without added metal driers. In preliminary evaluations, all coatings withstood direct and reverse impact as well as severe bending and cutting actions. These results are indicative of excellent flexibility, resistance to chipping, adhesion to substrate, and cohesive film properties. Also resistance to mineral acid, alkali, detergent, and solvent were judged excellent.

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CRAMBE. Koert J. Lessman and W. Powell Anderson, Agricultural Experiment Station, New Mexico State University P.O. Box 3.BF NM 88003.

Crambe (*Crambe abyssinica* Hochst Ex. R.E. Fries) is a promising new oilseed crop for the United States. The value of crambe lies in the industrial usefulness of its seed oil *per se* and in the erucic acid (a 22-carbon-chain fatty acid) present in its seed oil, as well as the use of the by-product seed meal, residual after oil extraction, as a supplement in livestock and poultry feeds. Crambe is one of the richest known sources of erucic acid, with 55 to 60 percent of its seed oil glycerides consisting of erucic acid. Traditionally, companies within the United States have obtained erucic acid and oil containing erucic acid from the seed oil of rape (*Brassica napus* and *B. compestris*), and this seed oil has been obtained from foreign rapeseed-growing countries. However, current trends in most of these countries is toward the development of rapeseed varieties with oil of improved nutritional quality as a food for humans and a reduction in erucic acid content. Hence, the increased interest in crambe as an alternate seed oil crop and source of erucic acid. This paper presents the development of crambe in the United States as an oilseed crop from its first introduction in the 1940s to the present, with emphasis on its genetic improvement and the purification of its seed meal.

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JOJOBA–A CROP WHOSE TIME HAS COME. D. M. Yermanos, Department of Botany & Plant Sciences, University of California, Riverside, CA 92521.

The demand for a substitute for sperm whale oil and for lubricants to replace depleting fossil fuel reserves set the stage for a major effort to develop jojoba as a commercial crop. Jojoba, a perennial species of the Sonoran desert, has long been known to scientists for its drought resistance and for the versatile liquid wax extracted from its seeds. During the 1970s jojoba graduated from being a botanical curiosity and entered the real world of agriculture. Commercial plantings are now in progress in the U.S., Mexico, Israel, Australia, South Africa and other countries. The only source of seed now is the wild populations of the Sonoran desert which are hand harvested. Thus, the oil is high priced ($50–$55/gal) and in limited quantities. Prices are expected to become more competitive and supplies more plentiful as soon as production starts in the commercial plantations established. Practically all of the oil available at this time is used for cosmetic preparations. The real challenge for jojoba will be to penetrate the vast market of lubricants. This may not be too difficult, however, because with the rapid disappearance of fossil oils cheap sources of lubricants will also be lost. None of the new sources of energy now contemplated have lubricants as by products.

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MACADAMIA NUTS AS AN EDIBLE OIL SOURCE. R.V. Harris and Neil Macfarlane, Tropical Products Institute, Industrial Development Department, Culham, Abingdon, Oxon OX14 3DA, England.

Traditional sorting and grading procedures for confectionery macadamia nuts reject about 25% of the crop. Investigations have been carried out on behalf of a tropical developing country on the feasibility of using these reject nuts as a practical source of edible oil. The nuts were found to have an oil content of about 60% and a high proportion of the oil could be readily extracted using a small conventional screw expeller. The major fatty acids of the oil are palmitic (9%), palmitoleic (22%), stearic (3%), oleic (57%), linoleic (2%), arachidic (2%) and eicosenoic (3%) and it has an average I.V. of 74. The oil produced by the small expeller was light in colour and retained the pleasant flavor of the macadamia nut. The crude

oil can be used after clarification by simple settling, but without further processing, for frying or other edible purposes and proved to be very resistant to oxidative or lipolytic deterioration. The oilcake contained 33% crude protein, with high sulfur amino acid levels and a good lysine content. It is considered suitable as a protein supplement for non-ruminant diets.

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PURE BUTTERFAT, A SOURCE OF FAT IN FOOD FABRICATION. Hermann Timmen, Federal Dairy Research Center, D-2300 Kiel, West Germany.

Milk fat or butterfat is generally known and primarily consumed as a component of milk and milk products. Modern dairy technology, however, does not only engage in the improvement of traditional and development of new milk products, but also stresses the isolation of the different milk components for use as ingredients in fabricated foods. Pure butterfat, from which moisture and nonfat dry matter have, for the most part, been removed, is the best and most economic trade product in which form milk fat can be transported and stored. Methods and machinery are now available for the isolation of pure butterfat immediately from cream, skipping the churning process and the intermediate storage and handling of butter. A growing importance in the world trade, mainly for the manufacture of recombined dairy products, has prompted the International Dairy Federation to establish standards for anhydrous milk fat and related products. In the EEC the authorities try to reduce the huge surplus of milk fat by the sale at a reduced price of pure butterfat, to which sensory and chemical labeling compounds have been added, for use in fabrication of certain food. Research on the parameters, which characterize and influence quality and shelf life of anhydrous milk fat, has been conducted. Also implications and problems of the use of butterfat, especially for baker products, are discussed.

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SPECIES OF RAPESEED AND MUSTARD AS OIL CROPS IN CALIFORNIA. P.F. Knowles, Department of Agronomy & Range Science, University of California, Davis, CA 95616.

Introductions of the following species have been grown in California: *Brassica campestris* (turnip rapeseed); *B. napus* (rapeseed); *B. juncea* (oriental or brown mustard); *B. carinata* (Ethiopian mustard); *B. trilocularis* (sarson); *B. nigra* (black mustard); *B. hirta* (yellow mustard); *B. tournefortii* (rail); and *Eruca sativa* (rocket). The first four of these have shown most promise. All of the better introductions are spring or summer types, but are grown during the winter at Davis. They have been tested primarily under dryland conditions as substitute crops for wheat or barley. Cultivars of *B. campestris* and *B. napus* were included in strip tests on farmers' fields in 1977-78, 1978-79, and 1979-80. A small commercial acreage of Midas, a cultivar of *B. napus* was grown in 1978-79 and 1979-80. Results indicate that these two species will give about half the yield of wheat. Major problems requiring further study are: weed control with herbicides; fertilizer requirements; and reduction of seed loss during harvest. No introductions of *B. juncea* and *B. carinata* were found to have very low levels of erucic acid in the seed oil.

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A NEW CLASS OF NITROGEN/SULFUR-CONTAINING HETEROCYCLIC FATTY ACID DERIVATIVES. S.M. Osman, Fasih Ahmad and Nasirulla, Section of Oils and Fats, Dept. of Chemistry, Aligarah Muslim University, Aligarh-202001, India; and W. Pimlott, University of Göteborg, Sweden.

Although fatty acids as chemical intermediates are being used, the recent emphasis on the industrial use of fatty chemicals as renewable resources relative to petrochemicals has attracted attention toward the synthesis of new fatty acids derivatives. Aimed at this objective, a PL-480 Project is being undertaken to synthesize hitherto unknown sulfur/nitrogen-containing heterocyclic fatty acid derivatives (oxathiolanes and thiazolidinones), as these compounds are known to possess interesting pharmacological and antibiotic properties. Five different long chain oxo-esters, methyl 10-keto-undecanoate; 12-keto; 9,10-diketo, 2-keto, and 3-keto octodecanoates were chosen as substrates in the present investigation. Condensation of β-mercaptoethanol in the presence of BF_3-etherate and acetic acid occurred with two oxo-esters resulting in the formation of S-heterocyclic derivatives characterized as methyl 10-(oxathiolane) undecanoate and methyl 12-(oxathiolane) octadecanoate. On the other hand, reaction of mercaptoacetic acid in the presence of ammonium carbonate took place in three oxo-esters affording long chain thiozolidionones characterized as methyl 10-(4'-thiazolidinone) undecanoate, methyl 12-(4'-thiazolidinone) octadecanoate and methyl 9(10)-oxo-10(9)-(4'thiazolidinone) octadecanoate. The structures of these heterocyclic derivatives have been supported by microanalysis and spectra data (IR, NMR, Mass).

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THE EXTRACTION, COMPOSITION AND PROPERTIES OF OIL FROM *CANDIDA CURVATA*. E.G. Hammond, Young Choi; Department of Food Technology, Iowa State University, Ames, IA 50011.

The fermentation of cheese whey and the ultrafiltered permeate from cheese whey by the yeast *Canidada curvata* to produce an edible oil has been suggested as a method of using whey. The yeast produces 50-60% oil, but its extraction from the yeast is difficult. A number of extraction methods were compared, and the best results were obtained by sequential extraction with 95% ethanol, hexane and benzene. After evaporation of the ethanol and benzene, the lipid that could be extracted with hexane was 80-90% triglyceride and residual complex lipids could be removed by degumming with water. The degummed oil contained negligible amounts of free fatty acids. The oil was 30.6% 16:0, 0.8% 16:1 11.9% 18:0, 50.3% 18:1, 6.0% 18:2, and 0.4% 18:3. The 18:1 and 18:2 were shown to be oleic and linoleic acids by infrared spectroscopy and ozonolysis. The complex lipids included phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidic acid. Squaene and sterols, but no tocopherols, were found in the nonsaponifiables. The stereospecific analysis of the triglycerides showed oleic acid concentrated on the *sn*-2-position and palmitic and stearic acids at the *sn*-1- and *sn*-3-positions. The oil melted between -10 and 22 C. The oil was quite stable to oxidation and required 20 days to reach a peroxide value of 3 meq/Kg at 55 C.

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ENVIRONMENTAL VARIABILITY IN OIL CONTENT AND COMPOSITION IN JOJOBA SEED *(SIMMONDSIA CHINENSIS)*. Alfredo J. Salazar-Zazueta, Centro de Investigaciones Agricolas del Noroeste (-CIANO-), Apartado Postal #515, Ciudad Obregón, Sonora, Mexico.

Jojoba is a perennial shrub, native of the Sonoran Desert, under study of domestication to yield an oil seed crop each year. Seeds collected from 45 different areas were analyzed for oil content and composition. From the 45 locations of jojoba plantings, 14 were in Israel and 31 were in Arizona. Oil content at the 14 sites in Israel ranged from 41.1% to 53.7% with a mean of 43.4%. Oil content at the 31 sites in Arizona varied from the mean average of 43.7 to 45.2% with a total mean of 44.5%. A significant positive correlation was found between seed weight and oil content of the seed. Its oil composition is mainly represented by a mixture of eicosenol (C 20:1) and docosenol (C 22:1). Throughout all samples analyzed the mean average percentage of eicosenoic acid (C 20:1) was over 35%. Oil content was analyzed by NMR and reported on a dry weight basis, while the oil composition was determined by gas liquid chromatography. The oil content and oil composition data are being used to compare the effect of genetic and environmental interactions on the variability of these traits in native jojoba planted in Sonora, Mexico.

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TALL OIL-THE MAJOR RENEWABLE RAW MATERIAL FOR FATTY CHEMICALS. E.E. McSweeney, Union Camp Corp. 1600 Valley Rd., Wayne, NJ 07470.

Fatty acids from natural, renewable sources have been a major raw material for important industrial chemicals for many years. The development of the tall oil distillation industry in the 50's gave great impetus to the use of unsaturated fatty acids with the result that tall oil is not only the major source of unsaturated fatty acids today, but has spawned the development of unique and important industrial chemicals. The history of this development is traced with emphasis on the current importance of tall oil fatty acids as a chemical raw material.

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THE OXIDATION PRODUCTS OF *TRANS*-3,5-DIMETHOXYSTILBENE. R.P. Scharrer and M. Epstein, Research Department, Arizona Chemical Company, 1937 W. Main St., Stamford, CT 06904.

trans-3,5-Dimethoxystilbene (I), a long-known tall oil fatty acid color body precursor, was oxidized with peracetic acid and subsequently chromatographed to give three discrete reaction products: 2-hydroxy-*trans*-3,5-dimethoxystilbene (II), 4-hydroxy-*trans*-3,5-dimethoxystilbene (III), and 2-hydroxy-5-methoxy-3-styryl-p-benzoquinone (IV), a dark red product. This last compound is probably responsible for the red color that develops when tall oil fatty acids containing I are epoxidized. These compounds were identified by elemental analyses, IR, NMR, and, in the case of IV, by single crystal X-ray defraction analyses. Additionally, two dark higher molecular weight fractions were isolated which appear to be oxidized oligomeric products of I. Peracetic acid oxidation of II indicates that it is the precursor to IV before the latter is oxidized to higher molecular weight products. Oxidation of III gives solely coupling products.

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A NEW FAMILY OF CHEMICAL COMPOUNDS HAVING AM-

PHOLYTIC PROPERTIES. John B. Braunwarth, Leonard J. Armstrong and Gerald A. Churchill, Armstrong Chemcal Co., Inc., 1530 S. Jackson St., Janesville, WI 53545.

A new family of chemical compounds having ampholytic properties has been invested. The compounds are produced by a condensation reaction involving tertiary amine compounds with ethylene oxide and acrylic acid. These compounds are characterized by their very good solubility properties, particularly in concentrated acids and bases. They have excellent foaming and foam stability properties over the entire pH range. Three select compounds from this group will be discussed with respect to wetting, surface tension and tergetometer studies. Other pertinent surfactant data will be presented. Toxicity data will also be furnished.

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A GPC-STUDY ON THE EFFECT OF MOLECULAR MASS AND CONTENT OF OLIGOMERS FORMED DURING HEATING OF DISTILLED FFA ON THEIR DISCOLORATION. Kryzysztof Krygier, Antoni Rutkowski, University of Warsaw (SGGW), ul. Grochowska 272, 03-849 Warszawa, Poland; and Jan Brzezinski, Institute of Industrial Chemistry.

The distilled fatty acids during their storage and transportation at temperatures above their melting point suffer from a considerable deterioration of quality, as revealed above all by a distinct discoloration and darkening. Results of our previous studies indicated that compounds responsible for these changes are oligomers formed during heating. To obtain a better insight in these phenomena the GPC-method was used for characterization of the products. Samples were analyzed with the Gel Permeation Chromatograph Model 200, Waters Associates, with a differential refractometer detector and a set of five columns filled with Styragel of 6.5×10^4, 10^4, 10^3, 10^2 and 10^2 A porosity. The system was calibrated with a series of polystyrene standards of narrow molecular mass distribution. Basing on this calibration, the content, number and weight average molecular masses as well as molecular mass distribution of oligomers was calaculated with a computer. Simultaneously, viscosity of samples and discoloration expressed as absorption at 450 nm of 25% solutions were measured. Chromatograms were obtained fro samples of a commercial mixture of distilled fatty acids, containing about 56% by weight of unsaturated fatty acids, after heating it during 5-15 days at a temperature of 50, 80 and 100 C. The existence of correlation between the color of the heated mixture of fatty acids and the overall content of oligomers (correlation coefficient CC = 0,870), their weight average molecular mass $\bar{M}_W$ (CC = 0,911) and number average molecular mass $\bar{M}_n$ (CC = 0,875) was established. E.g., in the mixture of fatty acids heated during 15 days at a temperature of 100 C, the content of oligomers was 46.8% by weight with $\bar{M}_W$ = 1780 and $\bar{M}_n$ = 1100; these oligomers contained about 15% by weight of molecules composed of more than 10 mers of fatty acid. Thus, the reactions of oligomerization of unsaturated fatty acids are decisive for the discoloration of fatty acids.

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ENERGY CONSERVATION AND ENVIRONMENTAL ASPECTS IN THE MANUFACTURE OF FAT-BASED CHEMICAL PRODUCTS. Dr. Gerhard Dieckelmann, Henkel KGaA, Postfach 1100, 4000 Düsseldorf 1, Germany.

The trend in present-day manufacture of oleochemicals is illustrated with the aid of three samples: (1) Disposal of waste chemicals in the oleochemical field, for example polymeric glycerine residues, bituminous fatty acid residues, filter aids etc., with simultaneous recovery of the heat of combustion; (2) special technologies to increase safety and reduce air pollution in a plant for the manufacture of fatty acid and fatty alcohol ethoxylates, with simultaneous recovery of ethylene oxide from the air; and (3) elimination of odors in the large scale manufactore of fatty acids and their derivatives.

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STORAGE EFFECTS ON THE LIPID OXIDATION SYSTEM OF PEANUTS. John A. Singleton, Harold E. Pattee, USDA, SEA, AR P.O. Box 5906, Raleigh, NC 27650.

Lipid oxidation in peanuts stored at two different moisture contents (6 and 9%) under controlled environment conditions of 4 C and 50% R.H. has been studied. Evidence of changes in the lipid fraction during storage was obtained by monitoring the volatile profile and the endogenous hydroperoxide content. High moisture (9%) peanuts produced a higher level hydroperoxides than low moisture (6%) peanuts by the end of the storage period–eight months. Observed changes in the neutral, glyco-, and phospholipid components during storage will also be discussed.

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COVALENT BINDING OF PEROXIDIZED PHOSPHOLIPIDS TO PROTEINS. H. Nielsen, Institute of Medical Biochemistry, Aarhus University, Aarhus, Denmark.

It was previously shown that peroxidized cardiolipin binds covalently to albumin (1,2) and γ-globulin (3). The covalent reaction between peroxidized phospholipids and proteins in general has now been investigated. Peroxidized cardiolipin (ox heart), phosphatidylserine (ox brain), phosphatidylethanolamine (egg) and lecithin (egg) all bind covalently to ovalbumin, albumin (human) and γ-globulin (bovine). Binding occurs only when the phospholipids are peroxidized. The amount of phospholipid phosphorus being bound to a particular protein is considerably higher for cardiolipin than for phosphatidylserine, lecithin and phosphatidylethanolamine. The ability of the individual peroxidized phospholipids to cross-link a protein intermolecularly differs greatly. Thus, polymerization of albumin by peroxidized cardiolipin is negligible, while half of the albumin is polymerized by peroxidized phosphatidylethanolamine under comparable conditions. 15-20% of the amino groups of albumin are consumed in the reaction with peroxidized phospholipids, and 27% are consumed when albumin is incubated with malonaldehyde. Blocking the amino groups of albumin (by maleylation) diminishes subsequent covalent binding of peroxidized cardiolipin with 50%. Therefore, amino groups appear to be essential for some of the binding. After reaction with peroxidized phospholipids, ultraviolet absorption of albumin is increased 3-8 times at its maximum (275-280 nm). The complex exhibit a characteristic fluorescence (excitation maxima at 314 nm and 355 nm; emission maximum at 405 nm) which is different from that of malonaldehyde-albumin complex. The general validity of covalent binding of peroxidized phospholipids to proteins as indicated in this report suggests that such binding may constitute an important way of protein damage in systems undergoing peroxidation.

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DECOMPOSITION OF POLYUNSATURATED HYDROPEROXIDES INDUCED BY METALS AND ANTIOXIDANTS. P.A.T. Swoboda and D.A. Hammond, A.R.C. Food Research Inst., Colney Lane, Norwich, NR4 7UA, England.

The decomposition of the first formed hydroperoxides of polyunsaturated fatty acids is an important reaction which affects both the increasing rate of autoxidation and the production of odorous cleavage compounds. We have studied, using thin layer chromatography and ultraviolet spectrophotometry, the effect on ethanolic solutions of isolated hydroperoxides of linoleate and linolenate of added copper or iron in the presence of α-tocopherol or butylated hydroxy toluene. The rate of decomposition of the hydroperoxides induced by copper was observed to be further accelerated by the presence of α-tocopherol. Whereas the reaction was catalytic for copper, a stoichiometric relationship was observed for tocopherol. The maximal rate of destruction of free fatty acid hydroperoxides was one hundred times faster than that for hydroperoxides of methyl esters. Other factors affecting these induced decompositions will also be described.

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FERRIC IRON DEPENDENT LIPID PEROXIDATION AS A FUNCTION OF pH. Steven D. Aust, Ming Tien and Bruce A. Svingen, Dept. of Biochemistry, Michigan State University, East Lansing, MI 48824.

Ferrous iron will catalyze a rapid rate of lipid hydroperoxide (LOOH) dependent lipid peroxidation with a broad pH optimum. Ferric iron also catalyzes LOOH dependent peroxidation of extracted microsomal lipid but the reaction is slower and exhibits a very sharp pH optimum of 2.2. Similar pH dependence was found by Barb, et al, (Trans. Faraday Soc., 1951, 47, 592-616) for ferric iron catalyzed decomposition of hydrogen peroxide. Lipid peroxidation required the presence of both LOOH and ferric ion. Using soybean lipoxidase to generate LOOH in liposomes, the rate of peroxidation was shown to be directly proportional to LOOH content. Initiation of peroxidation was found to dependent on ferric ion catalyzed LOOH breakdown. At high LOOH to ferric ion ratios, the rate of peroxidation was found to be directly proportional to ferric ion concentration. At low ratios, the reaction kinetics exhibit a more complex dependence on ferric ion concentration. Lack of inhibition by 20 mM mannitol or 0.2 mM 2,5-diphenylfuran indicates that neither hydroxyl radical nor singlet oxygen participates in the reaction. However, inhibition by 1 mM butylated hydroxytoluene in the reaction mixture indicates the mechanism is free radical.

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INFLUENCE OF LIPID PEROXIDATION ON PROTEIN AND LIPOPROTEIN SECRETION FROM ISOLATED HEPATOCYTES. Mario U. Dianzani, Enrico Gravela, Giluseppe Poli, Emanuele Albano, Elena Chiarpotto and Luciana Paradisi, Instituto di Patologia generale, Università di Torino, Corso Raffaello 30, 10125 Torino, Italy.

The pathogenesis of fatty liver has been studied in the past by using either whole animals or perfused livers. At the present, the use of isolated hepatocytes represents the new experimental approach to the problem. In the case of CCl_4-induced fatty liver, it has been

shown that isolated liver cells mimic the entire liver with regard to different responses to the poison. In fact, in isolated hepatocytes, as well as *in vivo*, CCl_4 stimulates lipid peroxidation, inhibits both protein synthesis and protein and lipoprotein secretion and induces fatty accumulation within the cell. Furthermore, studies with isolated liver cells whose protein or lipid were prelabelled *in vitro*, have emphasized the block in protein and lipoprotein secretion as the earliest mechanism of fat accumulation in CCl_4 poisoning. Carbon tetrachloride could impair the lipoprotein secretory pathway at different steps mainly by two major mechanisms, i.e. covalent binding of its metabolites to the liver lipids and proteins, or prooxidant effect on membrane lipids. To investigate the influence of the latter mechanism of damage in CCl_4-induced block in protein and lipoprotein secretion, CCl_4 poisoning of isolated liver cells has been carried out in the presence or in the absence of a free radical scavenger with a very strong antioxidant effect, promethazine. The actual implication of the lipid peroxidation mechanism in CCl_4-induced derangement of the secretory pathway is then discussed in relation to other experimental conditions of increased breakdown of membrane polyunsaturated fatty acids.

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AUTOXIDATION OF VEGETABLE OILS IN PRESENCE OF AMINOACIDS AND CARBOHYDRATES. Enzo Fedeli and Ada Gasparoli, Stazione Sperimentale Oli E Grassi, Via Giuseppe Colombo, 79-20133 Milano, Italy.

The influence of several products on the oxidation of fats is being studied. Amino acids, added to fats modify the autoxidation trend at elevated temperatures (180-200 C) inducing the formation of a minor amount of polymers, in comparison to oxidated products. Evidence is given on the formation of combination products between amino acids and fatty acids. Certain amino acids such as cisteine decompose generating radicals which intervene in the oxidation processes. Carbohydrates react also with fatty acids but their influence on the oxidation processes is low. Analysis of volatile materials coming from autoxidation in presence of the aforementioned substances consent to get information about the autoxidation mechanisms.

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FLUORESCENCE TEST OF OXIDATIVE ABUSE STATUS OF FATS, OILS AND DRY WHOLE TISSUE. William L. Porter, Ann Marie Wetherby and John G. Kapsalis, Food Sciences Laboratory, US Army Natick R&D Command, Natick, MA 01760.

A rapid, dry fluorescence method has been developed to assess the oxidative abuse status of fats and oils and dry tissue lipids. The method depends upon solid sample measurement of the fluorescence of compounds produced by reaction of the volatiles arising from peroxidizing lipids and the contaminant amines of a polyamide coating adsorbed on a glass or plastic plate as conventionally used in thin layer chromatography separations. No additional reagents are required and the reaction can occur at room temperature. Since the initiation is peroxidative, heat or certain metals (cobalt) accelerate it. With acceleration, the test can be completed in 30 minutes. The plate fluorescence is measured using a solid sample holder. It has an excitation maximum at 360 nm and an emission maximum at 425 nm. A 390 nm cut-off filter is used. Diffraction of the excitation beam causes a low intensity background pattern of bands. The fluorescence is presumed to arise from polymer-bound amino-iminopropene compounds resulting from the reaction of malondialdehyde and the known contaminant amines of the polyamide (poly-epsilon-caprolactam). Contact with the vapors from authentic malonaldehyde generators like acidified tetra-ethoxypropane generates the same fluorescence in the absence of peroxidizing lipids. Polyamide is essential. With lipids, the fluorescence can be excited and measured through glass and some plastics. It may be generated by contact either with the superjacent dry vapor phase, or with dry peroxidizing lipid, whether bulk triglyceride or polar lipid. The fluorescence can also be generated at normal ambient storage temperatures, permitting dry, in-packing monitoring of oxidation, either of bulk lipids (oils, fried potato chips) or dry tissue lipids (membrane of freeze-dried carrots). The method can be applied to the evaluation of antioxidants.

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FOOD QUALITY AS AFFECTED BY EXPOSURE TO ALDEHYDES FROM THE SECONDARY DEGRADATION PRODUCTS OF HYDROPEROXIDES. Salwa El-Magoli, Department of Food Science and Technology, Faculty of Agriculture, Cairo University, Giza, Cairo, Egypt.

The effect of aldehydes from secondary degradation of hydroperoxides was examined on the initation of lipid oxidation (El-Magoli and Karel, unpublished work). Hexanal and 2,4-decadienal greatly influenced lipid oxidation in a model system containing methyl oleate and linoleate. The effect of different storage conditions on the reaction of aldehydes with different lipids was investigated in relation to overall food quality. The nature of this reaction was studied in food systems containing different concentrations of lipids and stored under different conditions.

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EUROPEAN SAFETY STANDARDS. Allan Robinson, North Quay, Gladstone Dock, Bootle, Liverpool L201BG, England.

This paper will review the safety standards used in Great Britain and Europe for the design and operation of an extraction plant. The differences between European and United States standards will be noted.

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DEMONSTRATION OF THE HAZARDS OF HEXANE. L.J. Donnell, Vought Corp., P.O. Box 225907, Dallas, TX 75265.

This presentation is a demonstration showing the relative flammability of hexane in different concentrations of oxygen mixtures. Ignition of hexane in air will be made by flame, static electricity and heat. Comparison of explosive force using dust will be shown in relation to a flammable liquid.

ADDITIONS:

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CHINESE VEGETABLE FOOD COLORS. Kan Ching-hao, Cheng Mon-hua, and Lin Ching-yuan, Fukien Normal University, Foochow, China. Institute of Polymer.

Historically, the Chinese have used various mineral and vegetable food colors. Many of them were nonpoisonous and effective at low concentrations (1:2,000 to 1:200,000). Among these coloring materials, most were of vegetable origin and had cheap and wide distribution. Some were fermentation products, such as wine dregs from red wine of Fukien Province (China). The present paper emphasizes the chemical analysis of these products (including spectral analysis) as well as animal feeding experiments with the products. Thirty-five vegetable colors were studied, most of them isolated as pure compounds, and others in pure forms, suitable for application. Most of them were nonpoisonous. Some of them were oil soluble, others were water soluble, but both with a wide range of spectrum. The present authors want to encourage some fermentation products, especially the red koji extract.

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CONCANAVALIN A-MEDIATED AGGLUTINABILITY OF BALB 3T3 CELLS GROWN IN MEDIA SUPPLEMENTED WITH VARIOUS PHOSPHATIDYLCHOLINE MOLECULAR SPECIES. Salvatore Ruggieri, Donatella Tombaccini, Anna Fallani, and Gabriele Mugnai, Institute of General Pathology, Viale Morgagni 50, 50134 Florence, Italy.

An increased concanavalin A-mediated agglutinability has been reported in several systems of transformed cells; however, the mechanism of this property of transformed cells is still incompletely defined. A deeper knowledge of this matter would provide information on the basic surface changes associated with the transformation. In order to determine whether the agglutination properties of cells may be influenced by lipids of cell surface, we have studied the concanavalin A-mediated agglutinability of Balb 3T3 cells after exposure to growth media prepared by using delipidated fetal calf serum supplemented with cholesterol plus dipalmitoyl-, 1-palmitoyl-2-oleoyl-, dioleoyl-, or 1-palmitoyl-2-linoleoyl phosphatidylcholines. The results of this investigation whos that incorporation of dioleoyl phosphatidylcholine enhances agglutinability of Balb 3T3 cells to a level equivalent to that found in SV40-transformed Balb 3T3 cells. On the other hand, no appreciable effect on Balb 3T3 cell agglutinability was induced by incorporation of 1-palmitoyl-2-oleoyl-, dipalmitoyl-, or 1-palmitoyl-2-lino-leoyl phosphatidylcholines.

P1

CONCENTRATION DEPENDENT EFFECTS OF STEROL INHIBITORS UPON THE PATTERN OF BRAIN STEROL BIOSYNTHESIS. Richard J. Cenedella, Department of Biochemistry, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

The present study examines the possibility that the qualitative effects of inhibitors of sterol biosynthesis in the brain are concentration dependent. The drugs U18666A, 3β (2-diethylaminoethoxy) androst-5-en-17-one·HCl, and AY9944, *trans*-1,4-*bis* (2-chlorobenzyl-aminomethyl) cyclohexane·2HCl, have been examined because both drugs markedly affect brain sterol metabolism and can alter the structure and/or function of the developing brain. Treatment of the young rat with U18666A produces chronic epilepsy and AY9944 leads to grossly abnormal myelin structure (Brain Res., *150*, 343, 1978; Soc. Neurochem. Mtg. 1977, abst. 1071). Cell-free supernatants of homogenized brains (22- to 24-day-old rats) were incubated with 2-^{14}C-mevalonate (40.8 mCi/mM) for 5 hours at 37 C. Lipids were extracted into chloroform:methanol (2:1); then squalene, lanosterol, desmosterol, 7-dehydrocholesterol, cholesterol, and sterol esters were separated by thin-layer chromatography. Lipids were extracted from the plates and their ^{14}C content measured. The qualitative as well as quantitative effects of U18666A and to a lesser extent AY9944 upon brain sterol formation from ^{14}C-mevalonate were highly dependent upon drug concentration. At levels of 10^{-7}M and greater, U18666A produced a major block in incorporation of ^{14}C into lanosterol and digitonide precipitable sterols, whereas it increased ^{14}C-incorporation into squalene. At 10^{-8} to 10^{-9}M, U18666A had little effect upon formation of any of these labeled lipids, but selectively decreased incorporation into cholesterol and reciprocally increased incorporation into desmosterol. AY9944 markedly inhibited ^{14}C-incorporated into squalene, lanosterol, and total sterols, but only at concentrations greater than 10^{-4}M. Between 10^{-4} and 10^{-7}M, the drug selectively decreased ^{14}C-incorporation into cholesterol and increased it into 7-dehydrocholesterol. These results indicate that at higher concentrations both U18666A and AY9944 can decrease sterol biosynthesis by inhibiting at sites prior to formation of lanosterol. Such inhibitions obviously resulted in an almost complete block of sterol formation. At lower drug concentrations inhibition occurred at specific sites late in cholesterol biosynthesis, whereas the less sensitive sites prior to lanosterol were no longer inhibited. In conclusion, the qualitative as well as quantitative effects of sterol inhibitors can be highly concentration dependent. Supported by grants from the American Epilepsy Foundation and NIH (NS 14446 and EY 02568).

P2

EXAMINATION OF SELF-ASSOCIATION WITH THE SELECTED ALIPHATIC COMPOUNDS. Józef Sjiwiok, Teresa Kowalska, Halina Czarniecka, and Barbara Korczak, Sliwiok, Institute of Chemistry, Silesian University, 9 Szkolna St., 40-006 Katowice, Poland.

A new method was established for comparison of self-association with the selected aliphatic compounds, taking advantage of the differentiated affinity of those substances toward chromatographic paper as a low-polar adsorbent. Six glycols were taken into consideration, namely: ethandiol, propandiol-1,2 and -1,3, and butandiol-1,3, -2,3 and -1,4, as well as an oleyl alcohol-oleic acid bicomponent system with the changing molar fraction ratio of both compounds. One glycol also used Whatman chromatographic papers nos. 1 through 4. By applying equimolar amounts of consecutive substances on the emploied sorbents, and then determining the obtained spot surface areas without conducting a "classical" chromatographic procedure, we managed to establish mutual, semiquantitative dependences between the discussed substances, which reflected their differentiated ability to self-associate. These results were compared with the others, obtained with the help of IR spectroscopy and dielectric permeativity measurements. Some models were suggested, aiming to explain the different methods of adsorption on chromatographic paper with glycols containing the first order and the second order hydroxyl groups in their structure.

P3

MICROBIOLOGICAL STUDIES INVESTIGATING MUTAGENICITY OF DEEP FRYING FAT FRACTIONS AND SOME OF THEIR COMPONENTS. M. Scheutwinkel-Reich, G. Ingerowski, and H.-J. Stan, Institute f. Lebensmittelchemie, Müller-Breslau-Str. 10, 1000 Berlin 12, Germany.

It has been shown by long term feeding experiments in animals that processed deep-frying fats may have deleterious biological effects. Of special toxicological interest are certain fat fractions which arise in considerable amounts during the deep-frying process and which can be isolated by analytical methods. The so-called "polar fraction" can be obtained by silica gel chromatography. It consists mainly of dimeric and polymeric triglycerides. The so called "oxidized fatty acid fraction" is isolated as the petroleum ether insoluble residue. The amount of this fraction present in a processed deep-frying fat serves as the legal basis for determining the acceptability of a given fat for use in Germany. The transesterification of both fat fractions yields a complex mixture in which hydroxy fatty acids can be detected by gas chromatography-mass spectrometry. Complementing the toxicological studies in animals was the Salmonella/Microsome mutagenicity test, according to B.N. Ames, in order to detect possible mutagenicity of the described fat fractions, as well as the mono-, di-, tri-, tetrahydroxy, and hydroperoxy octadecanoic acids as model substances. The results showed no mutagenic effects in the classical Ames-test with the tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538. At higher concentrations of test substance, however, limited test reliability was found due to direct toxic effects on bacterial growth. Through investigating toxicity with various non-modified bacterial strains and yeasts, toxic effects were observed with di-, trihydroxy, and with hydroperoxy octadecanoic acids.

P4

THE EFFECT OF ETHANOL ON THE CONSTITUENTS OF THE GASTRIC MUCOUS BARRIER. Bronislaw L. Slomiany, Amalia Slomiany, and George B.J. Glass, Gastroenterology Research Laboratory, Department of Medicine, New York Medical College, New York, NY 10029.

The effect of ethanol on the gastric "mucous barrier" and gastric mucosal constituents was investigated. Deranged gastric secretions were obtained by perfusion *in vivo* of Ghosh-Lai rat stomachs with 10%, 15%, and 20% ethanol in saline. The content of protein, immunoreactive albumin, and hemoglobin were determined on native perfusates. Lipids were extracted from dialyzed and lyophilized samples of ethanol perfusates and saline controls, separated into individual components by means of two-dimensional thin-layer chromatography, and compared. The content and composition of protein-bound carbohydrates was determined on the insoluble residues after lipid extraction. Significant increments in the level of glycolipids (glyceroglucolipids), cholesterol esters, glycerides, protein, immunoreactive albumin, and protein-bound carbohydrates were found in the ethanol perfusates. Six times greater quantitities of glycolipids, proteins, and immunoreactive albumins were found in 20% ethanol perfusates as compared to saline controls. Also, a ten-fold increase of protein-bound carbohydrates and neutral glycerides, and an 18-fold increase of cholesterol esters in 20% ethanol perfusates were detected. The glycolipid fractions consisted of neutral and sulfated glyceroglucolipids. The saline and 10%, 15%, and 20% ethanol perfusates were devoid of phospholipids and glycosphingolipids. The data indicate that the presence of ethanol in the stomach causes depletion of the mucous barrier and leakage of serum proteins into the gastric lumen. From the absence of phospholipids and glycosphingolipids in the perfusates, one may infer that the gastric epithelial membranes and vascular membranes remained intact. Also, the data indicate that the mucous barrier contains glycoproteins and considerable quantities of lipids, of which glyceroglucolipids are the most prominent components, but in which phospholipids and glycosphingolipids are not present. Supported by USPHS, NIH grants AM-21684-02 and AM-25372-01.

P5

FATTY ACIDS OF THE MALE REPRODUCTIVE TRACT OF THE DOMESTIC CRICKET (*ACHETA DOMESTICUS*) AND FIELD CRICKET (*GRYLLUS* SPP.). R.E.Worthington, U.E. Brady, J.E. Thean, and D.M. Wilson, Jr., University of Georgia Experiment Station, Experiment, GA 30212.

Linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3) have been reported to be essential for the growth and development of several species of insects. In *A. domesticus* it has previously been shown that linoleic acid is necessary for reproduction. Prostaglandin synthetase has also been demonstrated in this species, suggesting that 18:2 is converted to arachidonic acid and thence to PGE_2. Analysis of the fatty acids of the reproductive tract of *A. domesticus* revealed 16.4% 16:0; 13.0% 18:0; 19.1% 18:1; 39.0% 18:2; 3.0% 20:0; 1.6% 20:1; 6.0% 20:3; and 0.3% 20:4. In the *Gryllus* spp. the fatty acids consisted of 19.3% 16:0; 8.5% 18:0; 22.2% 18:1; 33.3% 18:2; 1.2% 20:0; 1.5% 20:1; 6.2% 20:3; and 1.2% 20:4. The 20_c polyenoic acids were identified as 5, 11, 14-eicosatrienoic acid and 5, 8, 11, 14-eicosatetraenoic acid. Identifications were based upon GC/MS (molecular ion), reductive ozonolysis, and by co-chromatography with authentic standards on Silar 10C, OV-225 and OV-275.

P6

A NEW ENERGY-SAVING PROCESS FOR THE PRODUCTION OF CRUDE OIL WITH EXTREMELY LOW ANISIDIN- AND PEROXIDE-NUMBERS. Thorsten Homann, Manfred Knuth, and Wolfgang Stein,* Fried. Krupp GmbH, Krupp Industrie-Und Stahlbau, P.O. Box 900 800, D 2100 Hamburg 90, Germany.

A new oil milling process is described, which basically consists of the well-known operations prepressing and solvent extraction. It differs from the normal process, however, in that whole seeds up to approximately 10 mm partical size can be fed into a specially designed screw press without any preceding milling or heating. The

crude oil leaves the press at average temperatures of 30 to 50 C; thus, it is of better quality. Also, the obtained presscake shows a much better extractibility than that obtained from usual pressing operations. As a consequence, extraction times can be cut to achieve the same residual oil content. A comparison between the different processes regarding energy consumption and investment cost is based on experimental data from a pilot plant. The layout of production plants will be discussed.

P7

ASSESSMENT OF HUMAN LUNG MATURITY IN UTERO BY TAPPING TEST. Eric J. Sing, Department of Obstetrics and Gynecology, Northwestern University Medical School, 333 E. Superior St., Chicago, IL 60611.

Phosphatidyl choline was a major lung surfactant. Insufficient development of the surfactant in neonates was often associated with the respiratory distress syndrome. A tapping test was developed and performed on 89 amniotic fluid samples of various weeks of gestation to assess the surfactant's practical value as a rapid means of conforming adequate surface activity in the developing fetal lung. Approximately 1 ml of fresh unspun amniotic fluid was placed in a 100X13 mm test tube and acidified with 1 drop of dilute hydrochloric acid (1:1, v/v). To the acidified solution approximately 1.5 ml diethyl ether was added, and the mixture was gently tapped with one finger for one minute. Bubbles formed at the lower part of the layer of ether. Bubbles that were not stable or broke down within one minute indicated lung maturity, and the test was considered to be positive. Stable bubbles forming at the junction of the aqueous and the ether layer indicated that the lung was immature, and the test was considered to be negative. Results of the tapping test (as the test has been named) were compared with those of the shake test and with the neonatal outcome. No respiratory distress syndrome was observed with a positive tapping test. The results of the tapping test indicated the maturity or immaturity of the fetal lung within three minutes. An unskilled person may perform this test at bedside, office, or in the laboratory. Any doctor, nurse, or midwife can perform this test without difficulty outside a hospital environment. The tapping test depends on total saturated and unsaturated fatty acids in lung surfactant. It may be possible that saturated fatty acids are surfactant in lung. The tapping test—being economical, reliable, fast and easy to perform—may replace the lecithin/sphingomyelin ratio test. In conclusion, the tapping test is useful in predicting the risk of neonatal respiratory distress.

P8

CHOLESTEROL AND CHOLESTERYL ESTER FATTY ACIDS IN HUMAN MILK. Richard M. Clark, Ann Ferris, Nancy Fey, and Robert G. Jensen, Department of Nutritional Sciences, U-17, University of Connecticut, Storrs, CT 06268.

Milk samples were collected between 9:30 and 12:00 AM from ten mothers who were at least 4 weeks postpartum. One breast of each mother was emptied with the aid of an electric breast pump. Total lipids were extracted in chloroform-methanol and weighed. The mean lipid content of the milk was 2.9 gm per 100 ml. Lipid classes were separated by preparative TLC and quantitated by GLC. The mean value per 100 ml milk was 18.1 mg for unesterified cholesterol and 4.6 mg for esterified cholesterol. The fatty acids esterified with cholesterol in moles percent were lauric, 1.4; myristic, 3.8; palmitic, 24.5; palmitoleic, 1.3; stearic, 7.7; oleic, 45.0; linoleic, 15.8; linolenic, trace; and arachidonic, 1.2. To our knowledge, this is the first time the fatty acids associated with cholesterol in human milk have been reported. The fatty acid distribution is similar to that observed in milk triglycerides, suggesting they originated from the same metabolic pool.

P9

FLUOROMETRIC STUDIES ON HUMAN AND BABOON LIPOPROTEINS. Jeanne E. Rudzki, Arthur W. Kruski,* and Paul M. Horowitz, Department of Pathology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284.

The binding of human and baboon high density lipoprotein (HDL), low density lipoprotein (LDL), and HDL protein components with the amphiphilic anionic detergent sodium dodecyl sulfate (SDS) was studied fluorometrically. The intrinsic fluorescence intensity of the protein and the fluorescence intensity of the hydrophobic probe 2-p-toluidinyl-naphthalene-6-sulfonate (TNS) were measured at SDS concentrations increasing from 0 to 50 mM. The changes in fluorescence intensity of TNS in the presence of human apo A-I reflect the known observations that human apo A-I possesses four discrete, high-affinity binding sites for SDS, and that the protein undergoes a conformational change in the presence of amphiphile concentrations greater than those required for binding to the discrete sites. The TNS fluorescence intensity changes of human apo HDL were similar to but less dramatic than those of human apo A-I. Human LDL, HDL, and apo A-II, as well as baboon LDL, HDL, apo HDL, and apo A-I exhibited TNS fluorescence data different from that of human apo A-I and apo HDL. The intrinsic fluorescence intensity changes were complementary with this TNS data. The differences in the TNS fluorescence behavior between human and baboon apo A-I are consistent with their known ligand-binding properties and may indicate different aggregative properties. In addition, these fluorometric approaches offer facile methods for studying interactions involving lipoproteins.

P10

DIETARY ARACHIDONIC ACID REDUCES FATTY LIVER, INCREASES DIET CONSUMPTION AND WEIGHT GAIN IN ETHANOL-FED RATS. Steven C. Goheen, Edward C. Larkin, Marcia Manix, and G. Ananda Rao, Box 151H, VA Medical Center, 150 Muir Road, Martinez, CA 94553.

When we fed rats an ethanol diet supplemented with arachidonic acid (20:4), liver triglycerides (TG) were reduced *ca.* three-fold. Young male Sprague Dawley rats (200 g) were fed *ad libitum* a liquid diet containing 34% of the calories as ethanol and 35% as fat (AA-), or a control diet in which ethanol was replaced by an isocaloric amount of dextrose (CA-). Two other groups of rats were fed the same diets except that the fat was supplemented with 7% 20:4 in the alcohol (AA+) and control (CA+) groups. After four weeks, rats in the AA- group had the lowest body weights (267 g, AA-; 326 g, AA+; 403 g, CA-; 415 g, CA+), the lowest liver weights (8.7 g, AA-; 15.1 g, AA+; 15.3 g, CA-; 15.3 g, CA+) and the highest hepatic TG content (68.4 mg/g wet weight of liver, AA-; 27.6 mg/g, AA+; 7.0 mg/g, CA-; 9.9 mg/g, CA+). Also, rats in the AA- group consumed the least amount of food during the last two weeks (e.g., on the 28th day: 56 ml, AA-; 93 ml, AA+; 100 ml, CA-; 106 ml, CA+). The fatty acid composition of liver TG in rats fed the alcohol diet was similar to that of diet fat. Levels of 20:4 and docosatetraenoic acid (22:4) in liver TG fatty acids from rats fed diets without arachidonate (AA-, CA-) were low (trace to 1.6%). On feeding arachidonate, 20:4 increased to *ca.* 10% and 22.4 to *ca.* 5%. The content of liver phospholipids was not greatly altered by any of the four diets.

P11

VARIABILITY OF DAILY URINARY EXCRETION RATES OF PROSTAGLANDINS E_2 AND $F_{2\alpha}$ IN MALE SUBJECTS. Aldo Ferretti, Lipid Nutrition Laboratory, Nutrition Institute, Human Nutrition Center, SEA, U.S. Dept. of Agriculture, Beltsville, MD 20705.

Despite several investigations concerning changes, induced by diverse physiological conditions, in urinary excretion rates of primary prostaglandins (PG), there is a lack of published data on daily variations observable in humans under constant dietary, physiological, and environmental conditions. This deficiency—which makes the correlation of urinary PG levels with other variables under investigation uncertain—prompted us to establish individual baselines in a group of healthy volunteers. PGE_2 and $PGF_{2\alpha}$ concentrations in 24-hour urine specimens of five subjects, age 30 to 60, were measured at regular time intervals over a period of 3 to 5 months. Quantification was done by GC-MS-SIM and by use of deuterated internal standards. Our data show that the mean PG levels differ widely from subject to subject, as does the degree of scattering of individual data. It is unlikely that day-to-day dietary changes alone would significantly affect the activity of the renal PG system. The observed variations probably reflect cumulative effects of hormonal factors and of several other variables. These may include physical activity, emotional status, frequency of voiding, pH, and flow rate. Thus, evaluation of results from studies based on the assessment of renal PG synthesis must take this broad variability into account. We also determined that even 48-hour sexual abstinence prior to urine collection does not ensure absence of seminal fluid in the specimens. Indeed, we found that seminal fluid contamination (revealed by the presence of PGE_1) occurs more frequently than is generally realized—specifically in better than 30% of the samples processed by us. (We are grateful to Dr. U. Axen, the Upjohn Co., for generous gifts of tetradeutero-PGE_2 and tetradeutero-$PGF_{2\alpha}$).

P12

INVESTIGATION OF HYDROPHOBIC PROPERTIES OF HIGHER FATTY ALCOHOLS AND ACIDS. Józef Sliwiok, Bożena Kocjan, Janusz Szulik, and Teresa Kowalska, Sliwiok, Institute of Chemistry, Silesian University, 9, Szkolna St., 40-006 Katowice, Poland.

Investigation and comparison of hydrophobic properties with the selected aliphatic compounds was performed with the help of thin-layer chromatography. On the basis of visual effects, which were connected with the surface dissolving of chromatographic spots, the possibility of a relative comparison of the degrees of hydrophobic properties with the analyzed substances was mentioned. An aqueous solution of new fuchsine was applied as a visualizing agent. Substances under examination were the selected higher fatty alcohols and acids. The relative degrees of hydrophobic

properties were compared with solubility of the investigated substances in water. A proposal was made noting a mechanism reflecting intermolecular interactions between the water molecules on one hand and the aliphatic -OH and -COOH groups on the other. A thermodynamic evaluation of hydrophobic effects was presented as well.

P13

IDENTITY OF CHOLESTERYL ESTERS AND STRUCTURE OF PHOSPHATIDYL CHOLINES AND TRIACYLGLYCEROLS IN TYPES IIb AND IV HYPERLIPIDEMIA. Robert G. Jensen, Richard M. Clark, Leslie Gerhard, and Mark B. Fey, Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06268.

The fatty acid compositions of plasma cholesteryl esters (CEs) and the structure of phosphatidyl cholines (PCs) and triacylglycerols (TGs) from four healthy individuals, two patients with type IIb and three with type IV hyperlipoproteinemia (HLP) were determined. Very low density (VLDL), low density (LDL), and high density (HDL) lipoproteins (LPs) were isolated and the CEs, PCs, and TGs extracted. All CEs contained very little 18:0. The HLP-CEs had more 16:0, 20:4 and less 18:1 than normal CEs. In the *sn*-2 position of the PCs, the HLP-VLDLs contained less 18:0 and 20:4 than the normals, while the LDLs and HDLs had more saturates, 18:1 and less polyunsaturates. The composition of the *sn*-1 position was in some instances reversed. The total HLP-TG fatty acids had more 16:0 and less 18:1 than the normals and the distribution of fatty acids differed, with the IIbs varying the most.

P14

CONVERSION OF VISUAL TO INSTRUMENTAL METHODS OF MEASUREMENT OF YELLOWNESS OF OILS AND LIQUIDS. Richard S. Hunter, Hunter Associates Laboratory, Inc., 11495 Sunset Hills Road, Reston, VA 22090.

Yellowness is a characteristic of many oils and liquids. It is the result of a more-or-less natural tendency of many materials (especially organic) to absorb more light at the blue end of the spectrum than in the rest of the spectrum. The use of reproducible standards of colored glass or reagent grade chemicals as color references for visual comparison dates back into the 1800s. Instrumental methods of color analysis, on the other hand, are based on the known responses of human observers to wavelengths of light. These responses were standardized by the International Commission on Illumination (CIE) in 1931. Ron Stillman of the AOCS chaired an Inter-Society Color Council committee in the 1960s which developed the relationships of twenty-five of the older visual-comparison color scales to the instrumental CIE scales. Unfortunately, there was so much data in Stillman's 1962 report that a vehicle for its adequate publication was never found. For the present paper, the CIE data for three of the most widely used visual scales (Hazen or APHA, Gardner, Lovibond) have been taken and reduced to four established ASTM instrumental scales for yellowness (D1925, E313,D2244 b and b*). ASTM D1925, heretofore used primarily for plastics, appears to be the best suited for yellowness ratings of oils, resins, and other yellowish-to-amber materials.

P15

ISOLATION OF NERVONIC ACID FROM RAT ERYTHROCYTE LIPIDS BY THIN-LAYER CHROMATOGRAPHY. G. Ananda Rao, Steven C. Goheen, Robert L. Kilpatrick, and Edward C. Larkin, Box 151H, VA Medical Center, 150 Muir Road, Martinez, CA 94553.

We obtained pure nervonic acid ($C_{24:1}$) methyl ester from rat erythrocyte sphingomyelin, using various thin-layer chromatographic methods. Rat erythrocyte lipids contain glycero-phospholipids, cholesterol, and sphingomyelin. Glycerophospholipids were converted to fatty acid methyl esters (FAME) by alkaline methanolysis of total lipids from erythrocytes. Sphingomyelin was isolated from glycerophospholipid FAME and cholesterol by thin-layer chromatography on silica gel H using $CHCL_3$:CH_3OH:30% CH_3NH_2 (65:25:8 v/v). In this system, sphingomyelin migrated as two distinctly separate bands. Also, the FAME of sphingomyelin (prepared by acid methanolysis) separated as two bands on thin-layer chromatography on silica gel H plates with benzene as the developing solvent. In both cases, the fast moving bands of sphingomyelin or FAME comprised esters of $C_{22:0}$, $C_{24:0}$, $C_{24:1}$, $C_{24:2}$, whereas the slow moving band contained esters of $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{20:0}$. Fast migrating species of FAME were further separated using argentation thin-layer chromatography. In this system, three bands were formed corresponding to methyl esters of saturated acids ($C_{22:0}$ and $C_{24:0}$), $C_{24:1}$, and $C_{24:2}$, respectively. Even when the total FAME from sphingomyelin were subjected to argentation thin-layer chromatography, the esters of monoenoic acids $C_{24:1}$ and $C_{18:1}$ separated from each other. Alkaline hydrolysis and acidification generated nervonic acid from the methyl ester derivative. These methods could be used to prepare radioactive nervonic acid to study the synthesis and metabolism of various sphingolipids.

P16

IMPORTANCE OF THE ΔR_M AND $\Delta \log t_R$ COEFFICIENT VALUES IN GROUP IDENTIFICATION OF HIGHER FATTY ALCOHOLS, HIGHER FATTY ACIDS AND THEIR ETHYL ESTERS IN TLC AND GC. Halina Czarniecka and Józef Śliwiok, Śliwiok, Institute of Chemistry, Silesian University, 9 Szkolna St., 40-006 Katowice, Poland.

A new approach toward group identification of higher fatty alcohols, higher fatty acids, and their ethyl esters was established, based upon a straight-line and parallel course of the following functions: $R_{MX,Y}$ = f/nC/ in TLC /where X,Y–the applied mobile phases–and nC–number of carbon atoms in a molecule of chromatographed substance–and log $t_{RX,Y}$ = f/nC) in GC /where X,Y–the applied chromatographic systems. This approach was also based upon the constant values of the following parameters: $\Delta R_{MX,Y} = R_{MX} - R_{MY}$ and $\Delta \log t_{RX,Y} = \log t_{RX} - \log t_{RY}$. A parameter that was newly introduced into group identification was a directional coefficient calculated for the obtained pairs of parallel lines. All identification parameters, i.e., ΔR_M and "a" in TLC, and $\Delta \log t_R$ and "b" in GC, are of a significant analytical importance and allow the correct determination of group identity of examined compounds.

P17

ELAIDIC ACID UPTAKE AND OXIDATION BY EHRLICH ASCITES TUMOR CELLS. Atif B. Awad, Department of Biochemistry, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

Elaidic acid ($18:1^{\Delta 9}$, *trans*) is introduced to the American diet through the hydrogenation of vegetable oils that contain the natural isomer, oleic acid ($18:1^{\Delta 9}$, *cis*). It has been estimated that *trans* fatty acids make up to 55% of fatty acids of some margarines used in the U.S.A. The uptake and oxidation of *trans* fatty acids were studied in normal cells by several investigators; however, these aspects of *trans* fatty acid metabolism have not been studied in tumor cells, despite the fact that tumor cells incorporate these fatty acids upon feeding *trans* fatty acids to tumor-bearing animals. The present investigation was designed to fill this gap. Elaidic acid was compared with its *cis* counterpart (oleic acid) in their uptake and oxidation by Ehrlich ascites tumor cells (EATC) at different time intervals. Stearic acid (18:0) was similarly examined in other experiments. The molar ratio of free fatty acid to albumin in the incubation medium of all experiments was 4:1. These studies indicate that elaidic acid is taken up by EATC at the same rate as stearic acid. Stearic acid was taken up to a greater extent than oleic acid. The oxidation of these fatty acids to CO_2 followed the same pattern as in the case of the uptake studies, i.e., elaidic acid was oxidized at the same rate as the stearic acid but higher than oleic acid. The incorporation of these fatty acids into EATC lipids was different. Whereas stearic acid was not esterified readily in cellular phospholipids and triglycerides, elaidic acid, which has the same rate of uptake and oxidation as stearic acid, was esterified in phospholipids and triglycerides at a faster rate than stearic acid. These data indicate that tumor cells handle elaidic acid differently from normal cells in some of their aspects of metabolism. (Supported by NIH grant no. CA-21857).

P18

MAMMARY DYSPLASIA: α-TOCOPHEROL THERAPY, SERUM HORMONES AND LIPIDS. G.S. Sundaram, R. London, D. Strummer, P.P. Nair, S. Margolis, and P. Goldstein, OB & GYN Endocrine Research Laboratory, Sinai Hospital of Baltimore, Inc., Belvedere Ave. at Greenspring, Baltimore, MD 21215.

While treating ten patients with mammary dysplasia with α-tocopherol (600 I.U. daily for 8 weeks), we investigated its effects on serum cholesterol (Ch) and lipoproteins (LP). Estrogens were measured by radioimmunoassay; enzymatic methods were used to assay free and total Ch in low (VLDL + LDL) and high (HDL_2 & HDL_3) density LP, isolated by precipitation and ultracentrifugation. Remission of mammary dysplasia was observed in eight patients. During therapy, mean serum estriol levels rose from 0.81 to 1.23 ng/ml ($P<.01$) while estradiol fell from 0.59 to 0.43 ng/ml ($P<.05$) in the ten patients but not in four controls. In the patient group the ratio of total serum Ch to HDL Ch decreased from 5.3 to 4.3 ($P<.05$). Total serum Ch did not change significantly in either group; HDL_3 Ch increased from 20 to 27 mg/dl ($P<.05$) in the patients only. VLDL + LDL free Ch increased significantly ($P<.02$) in both patients (40 to 52 mg/dl) and controls (38 to 56 mg/dl). Thus, α-tocopherol not only promoted remission of mammary dysplasia, but also shifted Ch and LP patterns in a direction associated with a lower risk for cardiovascular disease. At least some of the changes in LP pattern and composition may be mediated by altered ratios of estriol to estradiol.

P19

NEUTRAL AND ACIDIC GLYCOSPHINGOLIPIDS OF GUINEA PIG GASTRIC MUCOSA. K. Kojima, A. Slomiany, V.L.N. Murty, N.I. Galicki, and B.L. Slomiany, Gastroenterology Research Laboratory, Department of Medicine, New York Medical College, New

York, NY 10029.

Mucosa scrapings from guinea pig stomachs were used to study the composition, species specificity, and content of neutral and acidic glycosphingolipids. Following chloroform/methanol extraction and alkaline methanolysis, the glycosphingolipids were separated on DEAE-Sephadex into neutral and acidic fractions. Separation of the acidic glycolipids into gangliosides and sulfated glycosphingolipids was achieved by column chromatography on silicic acid. The glycosphingolipids contained in each fraction were finally separated into individual components by thin-layer chromatography. The neutral glycosphingolipids were found to consist mainly of glucosylceramide (1.48 μM/g dry weight), galactosylceramide (0.84 μM), lactosylceramide (0.16 μM), digalactosylceramide (0.62 μM), and triglycosylceramide (0.72 μM). Lower quantities of Forssman glycolipid were also detected. None of the analyzed samples contained N-acetylglucosamine and fucose. The sulfated glycosphingolipids were represented by galactosylceramide sulfate (0.16 μM/g dry weight) and lactosylceramide sulfate (0.03 μM). Only traces of triglycosylceramide sulfate were detected. The ganglioside fraction (0.45 μM of sialic acid/g dry weight) consisted mainly of GM_3, GD_3, and G_7 gangliosides. Upon the treatment with neuraminidase, GM_3 and GD_3 were converted to lactosylceramide, whereas G_7 ganglioside gave galactosylceramide. Thus, the glycosphingolipids of guinea pig gastric mucosa differ from those of the other species, i.e., hog. Supported by USPHS, NIH grants AM-25372-01 and AM-21684-02.

P20

INCIDENCE AND SEVERITY OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN VITAMIN E DEFICIENT RATS. M.S. Calvo, D.L. Travis, and P.V. Johnston, 205 Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, IL 61801.

Prolonged vitamin E (VIT E) deficiency has been demonstrated to increase the fragility of erythrocyte membranes in humans and rodents. Since erythrocytes and lymphocytes share common origin in the pluripotential stem cells, one could also predict VIT E deficiency to alter membrane integrity and composition in those cells that mediate the immune response. Sheffy and Schultz (Cornell Vet. *68*:48, 1978) have demonstrated a depression in both antibody titres and proliferative response to mitogenic stimulation in VIT E deficient dogs. Other studies have repeatedly demonstrated the ability of supplemental VIT E to enhance humoral response in a variety of species (C.F. Nockels, Fed. Proc. *38*:2134, 1979). The present study was designed to examine the effect of VIT E deficiency on the course of experimental allergic encephalomyelitis (EAE), a putative animal model for multiple sclerosis (MS). Although differences between the disease and its model exist, victims of both demonstrate an abnormal immunologic state. The pathology of both MS and EAE is in part attributed to the infiltration of active demyelinating lymphocytes into the central nervous system (CNS). Two groups of weanling male Lewis rats were fed diets adequate or deficient in VIT E for 14 weeks prior to induction of EAE. Preliminary data based on both clinical symptoms and permeability of the CNS to ^{125}I-human γ-globulin indicate that VIT E deficiency exacerbates EAE in the Lewis rat. Alterations in the synthesis of prostaglandins (PG) may be involved, because PG of the E series are known suppressors of mitogenesis, and previous studies in this laboratory have demonstrated that aspirin (PG synthetase inhibitor) feeding prior to inoculation increased the severity of EAE in Lewis rats. VIT E deficiency-induced altered membrane composition and integrity may influence PG synthetase activity in lymphocytes and thus influence the infiltration of demyelinating cells into the CNS.

P21

EFFECT OF LONG TERM FEEDING OF DIETARY CHOLESTEROL AND SATURATION OF FAT ON BABOON HIGH DENSITY LIPOPROTEINS. Arthur W. Kruski, Glen E. Mott, and Henry C. McGill, Jr., Department of Pathology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284.

Seventy-six juvenile baboons were reared on four diets after weaning: 20% saturated fat (SF, P/S = .26) with or without 1 mg cholesterol (C)/Kcal; 20% unsaturated fat (UF, P/S = 2.14) with or without 1 mg C/Kcal. Serum C concentrations in high density lipoproteins (H) as well as very low density + low density lipoproteins (V+L) were determined by dextran sulfate-$CaCl_2$ precipitation at 3 years of age. The mean H/V+L C in mg/dl by diet groups were:

Type of dietary fat	Dietary cholesterol	
	Low	High
UF	62/48	68/87
SF	68/55	77/105

by analysis of variance, dietary C significantly elevated the C concentration of both V+L ($P<.01$) and H ($P<.05$). Dietary SF significantly elevated the C concentration of H ($P<.01$), but its effect on V+L was borderline ($P\sim.1$). There was no interaction between dietary C and SF. Under these conditions, dietary C has a much greater effect on V+L than does SF. In contrast, SF has about the same effect on the C concentration of H as does dietary C.

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THE INFLUENCE OF AN ASSOCIATION TYPE ON THE NATURE OF SECONDARY AUTOXIDATION PRODUCTS WITH SEVERAL *CIS*-OCTADECENE-9 DERIVATIVES. Józef Rzepa, Józef Śliwiok, Janusz Szulik, and Bożena Kocjan, Institute of Chemistry, Silesian University, 9, Szkolna St., 40-006 Katowice, Poland.

A strong influence of a functional group was established on the rate of autoxidation as well as on the formation of certain types of hydroperoxide associates, while examining the autoxidation process with a number of *cis*-octadecene-9 derivatives (oleyl alcohol, oleic acid and methyl oleate). These associates undergo decomposition according to different reaction patterns, which is reflected in the obtained secondary products. On the basis of our investigations and the results, four possible models of associative interactions through hydrogen bonds were suggested, each of them involving hydroperoxidic functional group and promoting a specific mechanism of decay toward various secondary products.

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TIRTIAUX FRACTIONATION: THE FLEXIBLE WAY TO NEW FATS. Alain Tirtiaux, S.A. Fractionnement Tirtiaux, 601, Chaussée de Charleroi, B-6220 Fleurus, Belgium.

Natural fractionation provides a simple and economical solution to the problem of splitting most edible fats and oils into several products. The Tirtiaux process, with its accurate crystallisation control, and long industrial experience, allows a choice of crystallisation conditions and separation temperatures to ensure obtaining products of specific quality at low cost. This is the result of a technique developed by Tirtiaux that consists mainly of the formation of suitable crystal seeds and the control of their growth by regulating the heat transferred from the fat to the coolant. The choice of the separation temperature and the ability to refractionate any one of the end-products gives a wide range of possible qualities. The separation is done on the Tirtiaux Florentine continuous vacuum filter equipped with a stainless steel perforated belt as filtration support. A recycling device for any crystals sucked through the belt at the edge of the horizontal vacuum surface ensures a filtration on a preformed cake. The coarse mesh of the belt, together with the large size of the crystals obtained, allow an easy filtration with low vacuum even if the viscosity of the oil is high. The filter is therefore able to operate on delicate crystals such as those obtained when fractionating hydrogenated soyabean and fishoil or when refractionating palm olein at low temperatures.