Meetings

Technical Paper Abstracts

Session A Food Safety Regulations Monday a.m.

1

HISTORICAL OVERVIEW OF FOOD SAFETY ENFORCEMENT, Sanford A. Miller, Director, Bureau of Foods, Food and Drug Administration, HFF-1, 200 C. Street, S.W., Washington, DC 20204.

Contemporary American food safety policy is a result of long and often painfully laborious evolutionary processes that began in the 18th century and reached its first milestone in 1906 with the passage of the first Pure Food and Drug Act. Starting as a statute designed primarily to deal with economic fraud, for the past 75 years this law has, with each modification, gradually emphasized increasing concern of the American people for the impact of food on their health. This concern reached its peak in 1958 with the inclusion in the food safety amendments of that year of the now famous Delaney clause. In spite of the evolutionary nature of American food law, the process has not been smooth. The law has been modified to reflect contemporary concerns with food, each modification engendered by a particular sense of crisis. In addition, changes in contemporary science have also led to a recognition that changes in law are required. Nevertheless, a careful analysis of the underlying issues involved at each decision point suggests that we are constantly encountering the same questions. What changes are the answers will depend upon contemporary societal standards as well as available scientific understanding. Today we are in the middle of another evolutionary critical point. A substantial review of current food safety policy is underway. This paper will consider both the historical and contemporary dynamics of food safety policy and try to suggest some of the future directions.

2

NEW APPROACHES TO THE REGULATION OF CARCINOGENS IN FOOD. Herbert Blumenthal and Robert J. Scheuplein,* Food and Drug Administration, HFF-150, Bureau of Foods, 200 C Street, S.W., Washington, DC 20204.

Food additives are now almost essential to the supply, storage, distribution and palatability of our food, and contaminants are the unavoidable residues of new technologies and economies of scale in methods of food production. The wider distribution of additives and contaminants coupled with the increased sensitivity of methods of chemical analysis occurring during the last 2 decades have generated new problems in the evaluation of the safety of food. One of these concerns is the potential risk of cancer from exposure to very low levels of substances found carcinogenic or mutagenic in laboratory studies. The problem is not new, but it is beginning to be realized that previous solutions to it, namely the total elimination of carcinogens at all levels from our food supply, as exemplified by the Delaney clause, are no longer practicable. Quantitative risk assessment has played a key role in the agency's attempts to deal with these problems where it is not proscribed by the statute. The limitations and virtues of quantitative risk assessment in the context of food safety will be discussed.

3

OVERVIEW OF THE "RED BOOK"—TOXICOLOGICAL PRINCIPLES. Alan M. Rulis, Food and Drug Administration, Bureau of Foods, HFF-330, 330 C St., S.W., Room 1042, Washington, DC 20204.

In October 1982, the FDA made available for use and comment, its Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food (the "Redbook"). The document presents a guideline for the safety review of new food additives, including assignment of additives into "concern levels." It also provides "decision elements" for defining the endpoints of the safety review process, and guidelines for performing the common toxicological studies. The document also presents a framework for objectively ranking all regulated additives over time, according to concerns related to changes in use patterns, or new toxicological data. The Redbook itself will be described, and comments so far received will be discussed. Also described will be results of ongoing work in applying the document's framework of principles to currently regulated food additives.

4

STATUS OF LEGISLATION TO REVISE THE FOOD SAFETY LAWS. Stuart M. Pape, Patton, Boggs & Blow, 2550 M Street, N.W., Washington, DC 20037.

Recent controversies over such widely used food ingredients as the artificial sweetener, saccharin and the preservative, sodium nitrite, have precipitated widespread reexamination of our nation's food safety laws. A broad consensus exists on the need to modernize the food safety portions of the Federal Food, Drug and Cosmetic Act. Notwithstanding the consensus, progress has been slow because the issue is politically difficult and no crisis exists in which the compelling need to enact changes is evident to the Congress. Previous efforts to revise the Act suggest, however, that the complexity of the issues, the involvement of many interest groups, and the competing demands on the time and attention of members of Congress make rapid amendment improbable. The Act needs to be revised because advances in toxicological testing, carcinogenesis, techniques of quantitative risk assessment and analytical chemistry have all outstripped the ability of the Food and Drug Administration to adapt its policies to contemporary scientific and technological reality. Failure to revise the law to permit food safety decisions to reflect current scientific knowledge and technological capability will diminish the credibility of the food safety regulatory process and frustrate desirable innovation in food processing and packaging technology. Appropriate and sensible changes in the law can be made, however, which will permit FDA to retain the ability to protect the public health from significant risks in the food supply, while also facilitating innovation. Four areas most need to be revised: first, the Act must incorporate a concept of safety that permits distinctions to be made between substances shown to present negligible (of no consequence) risks and others. Second, the absolute approach of the Delaney clause must be eliminated so that the regulation of carcinogenic additives can be based on science and, on the magnitude of the risk, if any. Third, the consideration of the health benefits of certain additives should be allowed so that a substance is not prohibited on the basis of an assessment of its risks without attention being paid to its health benefits. Finally, the food safety regulatory agencies must be given the flexibility to phase out the use of certain unique substances from the food supply if that result is warranted, over a period of years.

Session B Panel Discussion on Higher Education in Fats, Oils and Lipids Monday a.m.

No abstracts.

Session C Structure and Function of Protein Monday a.m.

5

RECENT ADVANCES IN THE STRUCTURAL DETERMINATION OF PROTEINS. Kenneth J. Wilson and James E. Strickler, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608.

The availability of protein structural information is an important asset to modern molecular biology. Primary sequence information is used for: (a) synthesizing oligonucleotide probes used in the identi-

Unless indicated by *, first name given is speaker.

fication and cloning of mRNAs as well as the identification of c- and g-DNA clones; (b) determining processing regions of either native or cloned products; (c) confirming the sequence of cloned proteins and (d) identifying sites of posttranslational modifications. In most cases, the amount of sample available for obtaining such information is limiting, hence efforts have been directed toward increasing the sensitivity of the methods used in protein isolation and sequencing. Recent improvements in high performance liquid chromatographic systems have been instrumental in the success of these developments. In laboratories specializing in microlevel characterizations, such systems are used for protein isolations, analytical and preparative separations of chemically and enzymatically derived fragments, amino acid analyses and identification of amino acid derivatives arising from step by step chemical degradation of the polypeptide chain. Similarly, the instruments used for carrying out these degradations have been improved through reductions in volumes, using alternative chemical methods (gas-phase deliveries, substitution of other isothiocyanates), and improvements in chemical purity. At the same time, alternative approaches have been developed for specifically cleaving proteins into more manageable fragments. This paper will discuss system requirements for microscale structural determination of peptide and proteins and will present specific applications of these techniques towards solving problems of interest in today's biotechnology field.

6

CHEMICAL MODIFICATIONS OF PROTEINS. Robert E. Feeney and John R. Whitaker, Department of Food Science and Technology, 1480 Chemistry Annex, University of California, Davis, CA 95616.

Chemical modifications of proteins present special problems because of the sensitivities of proteins to the harsh conditions used in many chemical reactions. In addition, proteins have side chains with varying reactivities to any one reagent. The side-chain groups of amino acids that are most susceptible to chemical modifications are thio, thioether, amino, imidazole, guanidino, indole, phenoxy and carboxyl groups. Each of these groups has its individual pK and intrinsic reactivity to different reagents. In chemical modifications, advantage can be taken of these differences in intrinsic reactivities and pK's to control reaction rates and specificities. Modifications are done for different purposes, including analyses for the side-chain groups, changing physical properties and changing biological activities by modifying active centers. Different types of special and sophisticated techniques are constantly being investigated. One type uses reagents known as "suicide" ones, which are relatively unreactive until converted to a reactive reagent by the enzyme's own catalytic process. These currently have extensive use in studies of enzyme active centers and show great promise for pharmaceutical applications. Chemical modifications are also caused by deteriorative processes during the handling and storage of foods and in animal tissues from disease or aging.

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NEW PROTEIN STRUCTURE-FUNCTION TECHNOLOGY AND APPLICATIONS. R.W. Gracy, Dept. of Biochemistry, North Texas State University/Texas College of Osteopathic Medicine, Camp Bowie at Montgomery, Fort Worth, TX 76107.

The recent advances in protein structure technology, e.g., affinity chromatography, high performance liquid chromatography (HPLC) for protein and peptide isolation and microsequencing, have provided new opportunities for answering fundamental questions as well as providing new applications. For example, for many years researchers have recognized that aging causes the accumulation of "abnormal" proteins in cells. The amount of these aged proteins in human cells is exceedingly small and only through the use of microstructural analyses have we been able to explore the properties of aged proteins. Such studies suggest that aged cells have a decreased ability to catabolize covalently modified proteins. High-sensitivity structural analyses also provide the opportunity to study the molecular basis of genetic diseases with small amounts of tissue or blood. Examples of the application of the new technology include the isolation of enzymes for therapeutic purposes such as the digestion of herniated spinal discs and the debridement of burns and ulcers. The use of microencapsulated proteins and matrix-bound enzymes has also provided new application opportunities. For example, covalently bound lactase has been found to have bio-adhesive properties in the intestine and may be used for treatment of lactase insufficiency in persons who cannot tolerate milk or milk products.

8

EFFECT OF PROTEIN CONFORMATION ON PROTEOLYTIC DIGESTIBILITY. C.N. Pace, Texas A&M University, Biochemistry Department, College Station, TX 77843.

Most of our quantitative knowledge of proteolytic enzyme hydrolysis is based on kinetic studies of small peptides. Consequently, much is known of the influence of covalent structures on the kinetics of proteolysis, but little information is available on the influence of conformation, since most small peptides do not form stable, folded conformations in an aqueous solution. We have used ribonuclease T_1 and its chemically modified derivatives as substrates, and trypsin as proteinase, to investigate the kinetics of proteolysis of a specific peptide bond in the folded and unfolded conformations of a protein. Steady state kinetic studies showed that $K_m = 0.27$ mM and $K_{cat} = 2.45\ s^{-1}$ for the tryptic hydrolysis of the Arg_{77}-Val_{78} peptide bond in unfolded ribonuclease T_1. This K_m is somewhat lower than, and the K_{cat} value similar to, values found for the tryptic hydrolysis of comparable bonds in small peptides. Our data for the initial velocity of hydrolysis of the Arg_{77}-Val_{78} bond in a solution of the folded protein indicate that the bond is at least 1,700 times less rapidly hydrolyzed in the folded than in the unfolded conformation of ribonuclease T_1, and do not exclude the possibility that the bond is completely resistant to hydrolysis in the folded protein. We will use these results to illustrate how the conformational stability of a globular protein affects its rate of digestion by proteolytic enzymes.

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CHEMICAL AND PHYSICAL STRUCTURES OF PROTEINS AND THEIR DIGESTIBILITIES. John R. Whitaker and Robert E. Feeney, University of California, Davis, Department of Food Science and Technology, 1480 Chemistry Annex, University of California, Davis, CA 95616.

Investigations of factors affecting the proteolytic digestion of proteins, whether in the gastrointestinal tract, the continuous metabolic turnover of proteins in tissues or in vitro studies, are of major importance to food science, nutrition, biochemistry, biology and medicine. In vivo under normal conditions, proteins have half-lives ranging from ca. 10 min for ornithine decarboxylase to years for elastin. Experimental evidence strongly indicates that the native protein is resistant to proteolysis, even though amino acid residues essential for recognition by a protease, e.g., trypsin, may be primarily on the surface of the protein. In vivo modification of proteins is known to affect their rates of turnover. An energy source appears to be required for proteolysis in some cases. Proteolysis leads, in some cases, to complete degradation; in other cases, proteolysis is limited to 1 or a few peptide bonds, frequently causing the formation of biologically active molecules. In the food industry, controlled proteolysis provides products with unique solubility and functional properties. Specific examples will be used to illustrate the relationship between chemical and physical structures of proteins, their digestion and the information that can be obtained from such studies.

Session D Membrane Filtration Technologies in Processing Monday a.m.

10

CROSSFLOW MEMBRANE FILTRATION—WHAT HAS BEEN DONE: WHAT DOES THE FUTURE HOLD? D. Dean Spatz, David J. Paulson and Leland F. Comb, Osmonics, Inc.

The membrane process of reverse osmosis, developed in 1959 by Sourirajan, is now 25 years old. The technology has moved from reverse osmosis being used for desalting water to include the cross-

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flow membrane systems of ultrafiltration for small protein fractionation, as well as newer uses of microfiltration membranes for larger protein separations. Ultrafiltration has now been used for commercial-scale protein fractionation for over 10 years. Substantial quantities of whey protein, blood protein, oil emulsions and latex emulsions are being processed on a daily basis. The future holds many opportunities for membrane separations of bio-fluids, including continuous fermentation. This paper will discuss the difference between reverse osmosis, ultrafiltration, microfiltration and particulate filtration. The primary emphasis will be on the use of membranes in crossflow filtration. Examples of equipment being used at present for crossflow filtration and some discussions of the limitations will be included. Potential new applications for crossflow membrane filtration will be presented and the audience will have the opportunity to see the vast potential this membrane technology holds.

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ULTRAFILTRATION, THEORY AND PRACTICE. Barry R. Breslau and Richard A. Aust, Romicon, Inc., 100 Cummings Park, Woburn, MA 01801.

Ultrafiltration is a pressure-activated membrane-separation process. It differs from reverse osmosis, another pressure-activated membrane-separating process, in that it employs a more open or porous membrane that will not retain low molecular-weight species (i.e., species having dimensions similar to that of the solvent [water] molecule). Ultrafiltration membranes offer substantially higher capacities than reverse-osmosis membranes and yet can function at significantly lower pressures (typically less than 100 psi) as this type of membrane generally does not have to overcome a substantial osmotic pressure gradient in order to generate permeate. In addition to being able to concentrate macromolecules, ultrafiltration membranes can also be used to purify or fractionate solutions of micro- and macromolecules as well as solutions of micromolecules and suspended matter. This fractionating ability is the result of being able to separate various species by size differentiation alone, in much the same manner as a screening or sieving operation. Sugars, for example, can readily be separated from solutions of macromolecules or suspended matter with an ultrafiltration membrane. Looking at the process from another point of view, macromolecules such as enzymes can be readily recovered as well as purified of low molecular-weight contaminants via ultrafiltration. This technique is routinely used in the pharmaceutical and biological industry. This paper, which presents the fundamentals of ultrafiltration theory, will also present the practical aspects of ultrafiltration, with an emphasis on material balance considerations.

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CONCENTRATION OF SOY PROTEIN IN AQUEOUS SYSTEMS BY ULTRAFILTRATION. Grant H. Hartman, Jr., Mead Johnson & Company, 2404 Pennsylvania St., Evansville, IN 47721.

Concentration of soy protein in aqueous systems is generally achieved by processing procedures that insolubilize the protein fraction, followed by centrifugation to remove the soluble carbohydrate, salts and other low molecular-weight components. Ultrafiltration can be used to reduce the level of soluble low molecular-weight soy components without insolubilizing the soy-protein fraction. Soy-protein isolate can be prepared by the ultrafiltration of neutralized defatted soy-flake extracts. Ultrafiltration to ½ the original volume, followed by diafiltration with an additional volume of water, will remove sufficient soluble carbohydrate to increase the protein level above 90% on a solids basis. In this process, the protein yield is enhanced and the amino-acid composition is improved because the soy-whey protein is retained by the ultrafiltration step. Soluble carbohydrate also can be removed from lipid-protein emulsions by membrane filtration. Both the protein and fat content of lipid-protein emulsions prepared by aqueous extraction of ground whole soybeans increased as the volume was reduced by ultrafiltration. Soy-protein products prepared by ultrafiltration have improved nutritional value and improved functional characteristics, e.g., protein solubility, protein suspendability, viscosity, oral texture and emulsion stability.

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REVERSE OSMOSIS AND ULTRAFILTRATION ON STILLAGE SOLUBLES FROM CORN DRY-MILLED FRACTIONS. Y.V. Wu and K.R. Sexson, Northern Regional Research Center, USDA, ARS, 1815 N. University St., Peoria, IL 61604.

Yellow grits, flour, degerminator meal and hominy feed from corn were fermented to make alcohol. The stillage, after the alcohol was distilled off, was separated by screening and centrifugation into insoluble solids and solubles. The stillage solubles contained 0.036-0.080% nitrogen and 1.4-7.2% solids. Ultrafiltration (UF) of stillage solubles separated the solutions into permeate and concentrate fractions. Permeate from stillage solubles accounts for 85-95% of the original volume, 44-67% of total solids and 40-75% of total nitrogen. Reverse osmosis (RO) of UF permeate separated the UF permeate into RO permeate and RO concentrate fractions. The RO permeate accounts for 70-92% of the original volume, 5-15% of total solids and 5-21% of total nitrogen of UF permeate. Conductivity of some RO permeate fractions was lower than that of tap water. The conductivity of permeate and concentrate from RO of UF permeate increased with increasing solids and ash contents as RO proceeded. UF and RO of stillage solubles from corn dry-milled fractions appear to be an attractive method to separate the solubles into a large volume of permeate with low solids and nitrogen and a small volume of concentrate with high solids and nitrogen contents. The RO permeate may be reused as water or discharged without evaporation of the water in stillage.

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MEMBRANE PROCESSING GLANDLESS AND GLANDED COTTONSEED FLOUR EXTRACTS TO PRODUCE EDIBLE PROTEIN ISOLATES. J.T. Lawhon and E.W. Lusas, Food Protein Research and Development Center, Texas A&M University, College Station, TX 77843.

Edible protein isolates were produced from hexane-extracted glandless cottonseed flour and acetone-hexane-water (AHW) extracted glanded cottonseed flour by ultrafiltration (UF). A process employing a combination of UF with isoelectric precipitation was preferred for glandless cottonseed protein isolation. Nonstorage (NSP) extract was first separated by acid precipitation into curd and whey. The lysine-rich NSP whey was then added to storage protein (SP) extract and the mixture ultrafiltered to obtain a higher yield of SP isolate having a lighter color and increased lysine content. AHW-extracted flour extracts were processed with large-pore UF membranes (100,000 molecular weight cutoff [MWCO]) to remove the so-called catty odor and unpleasant bitter taste normally imparted to oilseed products by acetone-containing solvents. Isolates were also produced from AHW extracts by a 30,000 MWCO membrane and by acid precipitation for comparison with the 100,000 MWCO membrane isolate in triangle taste tests. Whipping capacity, emulsification capacity, oil and water absorption and heat gelation measurements made on the AHW isolate showed that it should be functionally acceptable for a variety of food uses.

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PILOT-PLANT PREPARATION OF A SOY TRYPSIN INHIBITOR CONCENTRATE. E.C. Baker and J.J. Rackis, Northern Regional Research Center, ARS/USDA, 1815 North University Street, Peoria, IL 61604.

A soy trypsin inhibitor (TI) concentrate was required in sufficient quantities to support a chronic lifetime (2-year) feeding study with rats. Starting with commercial, lightly toasted, defatted soy flour with minimum denaturation of the protein, pilot-plant procedures involving mixing, centrifugation, ultrafiltration (UF) and freeze-drying were developed to separate the TI fraction from the non-TI protein, sugars, ash and polysaccharides. The TI fraction was separated from the non-TI protein by a combination of salting-in and isoelectric precipitation. The same separation attempted, with a range of molecular weight cutoff of UF membranes was not successful. Sugars and ash were removed in a UF procedure in which the feed volume was maintained constant for 16 hr before concentration. All procedures were carried out at ambient temperatures, and the final UF concentrate was freeze-dried. The freeze-dried soy TI concentrates had an average purity of 35% TI.

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Session E Potentiality of Biotechnology to the Industry Monday a.m.

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BIOTECHNOLOGY AND THE FATS AND OILS INDUSTRY—AN OVERVIEW. James B. M. Rattray, Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Biotechnology in its earliest forms was applied through agronomic practices to improve the yields of oil-bearing plants. The oils so obtained have found uses as components of the diet of man or as chemical feedstocks for industrial purposes. Sources of novel oils and unusual fatty acid components continue to attract attention. Plant breeding programs and genetic engineering have been suggested as means of improving the quantity and quality of various oils, and presently form the bases of much research. Alternative biotechnological practices involve the applications of cell culture procedures and immobilized systems. Practical examples, as directly related to the fats and oils industry, are so far limited. Nonetheless, the commercial feasibility of using microorganisms for the production of speciality fats (e.g., as a cocoa butter substitute) and various fatty acids, waxes and surfactants has been considered in several cases. In addition, the exploitation of select enzyme systems has been made in a variety of relevant processes ranging from the facilitation of oil extraction of seeds and the interesterification of fats and oils, to flavor modification of fatty foods and the production of pharmaceutically valuable steroids. The potentiality of biotechnology in the fats and oils industry requires careful analysis with due regard being paid to the specific fatty product, engineering considerations, plant facilities and overall economics.

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PROGRESS OF IMPROVING THE FATTY ACID COMPOSITION OF SOYBEAN OIL. E. G. Hammond, Iowa State University, Department of Food Technology, Ames, IA 50011, and W. R. Fehr, Department of Agronomy, Iowa State University, Ames, IA 50011.

The development of soybean genotypes with improved oil quality has involved the production of 1 generation in Iowa during the summer and 2 generations during the winter in Puerto Rico. A recent comparison of the fatty acid composition of lines grown in the 2 locations indicated that selection could be practiced as effectively in Puerto Rico as in Iowa. A5, the low-linolenic acid line developed at Iowa State, was grown in the same environment as a mutant line developed at Purdue, which has significantly lower linolenic acid than its parent cultivar—Century. A5 oil had ca. 1% lower linolenic acid than the Century mutant. The lines have been crossed to determine if progeny with lower linolenic acid than either parent can be obtained. Seeds of A5 have been treated with ethyl methanesulfonate and sodium azide in an attempt to obtain lines with even lower linolenic acid. A hybridization program has been initiated to transfer the genetic factors for low linolenic acid in A5 to high-yielding cultures. Sufficient oil of A5 with less than 3% linolenic acid was produced to evaluate some of its properties. It had increased stability and a lower phosphorus content than oil with above 6% linolenic acid from the cultivar, Pella. Three independent mutants have been found with an elevated stearic acid percentage. The mutations seem to represent different recessive alleles at the same locus.

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NOVEL PALM OILS FROM CLONED PALMS. L. H. Jones, Unilever Research, Sharnbrook, Bedford, England.

The ability to propagate selected oil palms by tissue culture enables us not only to choose palms of exceptional yield potential, but also to establish clones bearing oil of widely different composition. Variability exists within the present populations of the West African oil palm, (*Elaeis guineensis* L.) and can be extended by hybridization with the South American species, *E. oleifera*. The first clonal oil palms, produced in 1975, have now been bearing fruit for several years and their characteristics have been determined. The performance of these clones in terms of uniformity, yield and oil composition has been considered. The prospects for the industry of novel types of palm oil from cloned palm varieties have yet to be established. I shall discuss the performance of these clones in terms of uniformity, yield and oil composition. In conclusion, I will examine the prospects for the industry of novel types of palm oil from cloned palm varieties.

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COCOA BEAN CELL AND EMBRYO CULTURE. Ming-che Wen, Bruce German and John E. Kinsella, Institute of Food Science, Cornell University, Ithaca, NY 14853.

Callus culture of cocoa bean was initiated from immature cotyledons on agar medium. By dispersing these callus cells, a liquid suspension culture was established. Lipid composition of cocoa suspension culture was investigated and was compared with those of cocoa beans of different maturity. Factors affecting fatty acid and triglyceride synthesis in cocoa suspension culture were also studied. Recently, asexual embryos were obtained from zygotic embryos of the white to white/pink stage of maturity cultured in semisolid or liquid medium. The factors affecting the lipid composition and metabolism in these cultured embryos are being studied.

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PROSPECTS AND PROBLEMS IN THE LARGE SCALE PRODUCTION OF METABOLITES FROM PLANT CELL TISSUE CULTURES. M. L. Shuler, J. W. Pyne and A. G. Hallsby, School of Chemical Engineering, 340 Olin Hall, Cornell University, Ithaca, NY 14853.

A wide range of plant products can be directly made by using plant-cell tissue cultures. However, the economic production of even high-value products from such cultures has not been commercially demonstrated. One problem is that rapid growth and high product yields often appear to be mutually exclusive with plant-cell tissue cultures. Some level of cellular differentiation is often required for the expression of genes associated with product formation; only dedifferentiated cells grow rapidly. Another problem results from the tendency of plant cells to form aggregates, which leads to a mixture of cell types in culture. The biological response is a function not only of the chemical environment but also the physical (e.g., hydrodynamic) environment, which makes scale-up of suspension processes difficult. In addition, cell lysis caused by high-liquid shear is a significant design constraint. Some of these problems can be circumvented by using multistage continuous culture devices or with immobilized cell reactors. Emphasis will be on membrane-entrapped cultures.

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EVALUATION OF APPLICATIONS OF PLANT-CELL TISSUE CULTURE. David W. Wheat, Alegria B. Caragay and Alejandro A. Herrero, Arthur D. Little, Inc., 15 Acorn Park, Cambridge, MA 02140.

Many applications have been proposed for the commercial use of plant-cell culture techniques. These fall in several categories, including application to advanced plant-breeding programs and the production of specialty and fine chemicals. In the latter category, applications to food ingredients, pharmaceuticals and industrial chemicals have been suggested. A list of proposed applications in these areas will be presented. Some of these proposed applications may be viable as commercial opportunities, while others will not be. Arthur D. Little, Inc. has been called on to evaluate the market potential and potential profitability of using plant-cell culture techniques in several areas. Such evaluations are essential for opportunity identification, research planning, capital investment decisions and market entry. The techniques we have used to answer such questions will be discussed with reference to selected categories of products. These will include both new chemicals, whose production is only made possible by the use of advanced tissue-culture methods, and existing chemical products, where cost reduction or reliability of supply is the object.

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PRODUCTION OF PROTEIN AND VOLATILE FATTY ACIDS IN THE FERMENTATION OF BLACKSTRAP MOLASSES BY EUBACTERIUM RUMANTIUM. Ketan I. Mehta, Anderson Clayton Foods, 3333 North Central Expressway, Richardson, TX 75080, and C. D. Callihan, Louisiana State University.

Production of protein and volatile fatty acids by anaerobic digestion of blackstrap molasses was investigated. This protein may have potential as a feed for animals (and, we hope, for human consumption) in the near future. Fermentation of blackstrap molasses by *Eubacterium rumantium* isolated from ruminant fluid was studied in a chemostat. A constant temperature of 37 C was maintained. This study focused on the kinetics of growth of the pure culture. The maximum rate of cell production was found when the pH and retention times were 6.2 and between 5 and 7 hours, respectively. The average cell yield was 12.6% and carbohydrate conversion was from 82% to 99%. Volatile fatty acids were also produced with acetic acid and n-butyric acid being the predominant products. Other volatile fatty acid produced was propionic acid. Six different kinetic models were used to fit the experimental data. The kinetic parameters obtained for the Monod model with decay constant were: u_{max} = 0.213 hour^{-1}, K_S = 0.136 g/l; and k_d = 0.007 hour^{-1}.

Session F Fatty Acid Synthases: Structure, Function and Molecular Biology Monday a.m.

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PRESENT STATUS CONCERNING THE BIOSYNTHESIS OF FATTY ACIDS IN HIGHER PLANTS. P. K. Stumpf, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616.

In recent years, the molecular characteristics of the fatty acid synthetase system (FAS) has been elucidated in higher plants. The FAS system is a nonassociated protein system very similar to the *E. coli* FAS system. In reconstitution experiments with purified proteins of the plant FAS system, it was shown that (a) varying the concentration of acetyl CoA:ACP transacylase markedly perturbed the final composition of the newly synthesized fatty acids; (b) β-ketoacyl ACP synthetase I is very sensitive to cerulenin and responsible for the synthesis of C_4–C_{16} fatty acids; (c) β-ketoacyl ACP synthetase II is strictly involved in the synthesis of stearoyl ACP from palmitoyl ACP. Discussion will also center around the microsomal elongation systems, which are responsible for the elongation of C_{18} fatty acid to very long chain fatty acids in a number of plant tissues. Integration of the ACP and the CoA-mediated systems will be considered.

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GENETIC STUDIES ON YEAST FATTY ACID SYNTHASE. Eckhart Schweizer, Michael Schweizer, L. M. Roberts and H. J. Höltke, Institut für Mikrobiologie und Biochemie, Lehrstuhl für Biochemie, Universität Erlangen-Nürnberg, Egerlandstr. 7, D-8520 Erlangen, W. Germany.

By combined genetic and biochemical studies, yeast fatty acid synthetase (FAS) was shown to have an $\alpha_6\beta_6$ molecular structure. Subunits α and β are multifunctional proteins of mol wt 185,000 and 180,000 daltons, respectively. Three FAS functions were localized on α (β-ketoacyl synthase, β-ketoacyl reductase, acyl carrier protein) whereas the remaining 5 (dehydratase, enoyl reductase and the 3 acyl transferases) are located on β. Studies on structural gene mutations of α (FAS 2) and β (FAS 1) revealed that the synthesis of both FAS subunits is coordinated, that all domains except the 3 acyl transferases are structurally independent parts of the 2 multifunctional FAS subunits and that the minimal functional protomer of FAS is the dimer $\alpha_2\beta_2$. A reaction mechanism is proposed that is strictly dependent on this structure. Recently, the yeast fatty acid synthetase genes FAS 1 and FAS 2 were cloned into the novel yeast cosmid shuttle vector, pMS 201. In these clones, FAS 1 is contained in 17 kb, FAS 2 in 25 kb of contiguous DNA. By transcription mapping, the length of the FAS 1 coding region was determined as 5–6 kb. A detailed restriction map of this area was constructed together with a large collection of FAS 1 subclones. Transformation studies using subcloned FAS 1 DNA segments revealed the relative locations of several catalytic domains within the pentafunctional cluster gene. These studies are extended using the large collection of FAS 1 and FAS 2 mutants available to us. In addition, parts of the sequence of FAS 1 and its surrounding DNA will be presented.

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STRUCTURE OF YEAST FATTY ACID SYNTHETASE AND CHARACTERIZATION OF SUBUNITS' GENES. Michael A. Kuziora, Debra M. Spector, James K. Stoops and Salih J. Wakil, Biochemistry Department, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Fatty acid synthetase from *Saccharomyces cerevisiae* is a complex of 2 multifunctional subunits: α (M_r 212,000) and β (M_r 203,000). The native enzyme has a molecular weight of 2.4 × 10^6 or an $\alpha_6\beta_6$ structure. Electron microscopic studies showed the enzyme as an ovate molecule containing on its short axis, plate-like structures (α subunits) to which archlike structures (β subunits) are attached and equally distributed on either side. Recent studies involving the inhibition of enzyme activity by various alkylating agents yielded information in support of this model for the yeast synthetase and the proposal that a palmitate synthesizing unit is constructed from a β subunit and 2 complementary halves of 2 adjacent α subunits. The genes encoding the yeast synthetase subunits have been cloned using 2 independent methods. Plasmids YEpFAS1 and YEpFAS2 were isolated by complementation of FAS 1 and FAS 2 yeast strains, respectively. Plasmids 33F1 and 102B5 were isolated by immunological screening. Restriction mapping and Southern blotting experiments showed that YEpFAS1 and 33F1 contain a homologous segment of yeast DNA, whereas 102B5 was found to be homologous to YEpFAS2. Complementation studies of FAS 1 mutations with YEpFAS1 or YEp33F1-21 (a subclone containing the entire yeast DNA insert of 33F1 in the vector YEp13) showed that the yeast DNA contained in 33F1 can complement mutations throughout the FAS 1 gene whereas YEpFAS1 can only complement a mutation in the enoyl reductase activity. These observations suggest that YEpFAS1 does not contain the entire FAS 1 gene. Further studies on the mechanism of how YEpFAS1 can complement an enoyl reductase mutation should reveal information on the structure of this multifunctional enzyme.

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MAMMARY GLAND FATTY ACID SYNTHASE. Stuart Smith, Bruce Lyon Memorial Research Laboratory, Children's Hospital Medical Center, 51st and Grove Streets, Oakland, CA 94609.

Mammary gland fatty acid synthesizing systems can be classified into 3 distinct types: (a) those that synthesize only long-chain fatty acids (C_{16}) via the fatty acid synthase (FAS), (b) those that synthesize short- and medium-chain fatty acids (C_4–C_{12}) using a transacylase function of the FAS and (c) those that synthesize medium-chain fatty acids via FAS and a separate chain-terminating thioesterase enzyme. The synthesis of long-chain fatty acids by the first type of system is regulated by a thioesterase activity associated with a covalently linked domain of the FAS. This thioesterase terminates growth of the acyl chain by hydrolyzing the C_{16} acyl moiety from its thioester linkage to the 4'-phosphopantetheine prosthetic group. This chain-termination mechanism, which results in the release of free palmitic acid, is common in the fatty acid synthesizing systems of most animal tissues. Work in Knudsen's laboratory has shown that ruminants synthesize shorter-chain products by a different mechanism, involving the transacylase, rather than the thioesterase, component of FAS. Whereas in nonruminants only C_2 and C_4 acyl moieties can be transferred between CoA and enzyme, ruminant FAS can transacylate C_2–C_{12} moieties. Thus ruminant FAS can release short- and medium-chain acyl moieties from the enzyme as CoA esters, rather than free acids. The third type of fatty acid synthesizing system is found in the mammary glands of non-

ruminants (e.g., rats, mice, rabbits and humans) and involves participation of both the FAS and an additional regulatory thioesterase enzyme, which is not an integral part of the FAS. This thioesterase, a monomer of 32,000 daltons with a single active-site serine residue, is able to interact with the 4'-phosphopantetheine of the FAS carrying the growing acyl chain and hydrolyze medium-chain acyl moieties from the enzyme. Thus, the products of this system are medium-chain free fatty acids.

Session G Hydrogenation Monday p.m.

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EVALUATION OF CONTINUOUSLY HYDROGENIZED OILS FOR SALADS AND COOKING. K. J. Moulton, Sr., K. Warner, E. N. Frankel and T. L. Mounts, Northern Regional Research Center, NCR-ARS-USDA.

Soybean oil was hydrogenated in a continuous slurry system using copper and nickel catalysts. The products were evaluated for flavor and oxidative stability. Processing conditions were varied to produce oils of linolenate contents between 0.4% and 2.7%, as follows: oil flow (0.5–2.2 L/hr), reaction temperature (180–225 C), H_2 pressure (100–525 psig) and catalyst concentration (0.5–1% copper catalyst or 0.1% nickel catalyst). *trans* Unsaturation varied from 8.4% to 22.9% with copper catalyst and from 15.0% to 27.4% with nickel catalyst. Linolenate selectivity was 8–9 with copper catalyst and 2 with nickel catalyst. Flavor evaluation of the finished oils containing 0.01% citric acid conducted initially and after accelerated storage at 60 C showed no significant difference between the hydrogenated oils and nonhydrogenated (control) oil. However, the oxidative stabilities of the hydrogenated oils, based on peroxide values and induction periods, were superior to that of the nonhydrogenated oil. In another report at this meeting (Frankel et al.), sensory evaluations at frying temperatures (190 C) show a positive improvement with the partially hydrogenated oils.

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DETERMINATION OF IODINE VALUE BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY. N. M. Ingber and D. S. Kalka, Durkee Foods, SCM Corporation, 16651 Sprague Road, Strongsville, OH 44136.

The iodine value (IV) of hydrogenated fats and oils from 0 to 130 IV can be determined by near-infrared reflectance (NIR). Both hydrogenated soybean and cottonseed oils yield results to within 1.5 IV units of the lab values. The early sample inlet/outlet configuration prevented the analysis of oils in the 0 to 35 IV range because of freezing in the lines. Recent laboratory modifications allow for the analysis of low IV streams. The method of calibration consists of multiple regression techniques in conjunction with a microcomputer equipped with a permanent program for the operation of the instrument and for the development of the calibration coefficients. Two calibration sets were developed, i.e., 30 to 135 IV for hydrogenated oils and 0 to 30 for all others. The method is rapid and requires no sample preparation other than liquifaction and heating to 75 C. Once the calibration sets are entered into the instrument, readings can be obtained in ca. 45 sec. A sample of 25 to 30 mL is sufficient to flush the previous sample for a complete changeover in ca. 1 min. Repeatability tests show a standard deviation of ±0.32 for high IV hydrogenated oils, and ±0.76 for low IV oils such as palm kernel and coconut oils and soybean stearines.

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CAPILLARY GAS CHROMATOGRAPHY VOLATILE ANALYSIS OF HEATED FATS. EFFECT OF HYDROGENATION AND ADDITIVES. J. M. Snyder, E. N. Frankel and K. Warner, Northern Regional Research Center, 1815 N. University Street, Peoria, IL.

Volatile analysis of heated soybean oil was investigated to determine what effect hydrogenation and additives have on the formation of total and individual volatiles. Soybean oil was hydrogenated to varying linolenate contents with either nickel (Ni) or copper (Cu) catalysts. Oils were stabilized with citric acid (CA) or a combination of CA with tertiary butyl hydroquinone (TBHQ) and/or methyl silicone (MS). Volatiles were analyzed with a capillary gas chromatograph equipped with a head-space sampler positioned on the injector. To study thermal abuse and frying performance of oils, samples were heated for several hours with prolonged bread frying. The deterioration of the oils was further accelerated by static heating in air within the head space sampler. All hydrogenated oils, containing 0.4–4.6% linolenate (Ln), produced less total volatiles than the unhydrogenated control oil after prolonged heating and bread frying. Static heating at 190 C for 1 h showed that the oil hydrogenated with Ni to 0.4% Ln was the most stable. MS decreased the formation of volatiles in all samples and was particularly effective in stabilizing the hydrogenated oils. However, MS had little effect on volatile formation after aging the oil at 60 C for 8 days. TBHQ was only effective in reducing volatiles in the oil hydrogenated to 0.4% Ln with Ni. With both TBHQ and MS, further stabilization was observed with all oils, showing a synergistic effect. Unique volatile compounds identified included heptadienal in nonhydrogenated soybean oil and nonenal in all hydrogenated oils. On heating, the amount of heptenal decreased significantly in the Ni-hydrogenated oils compared with the control. Hexanal, on the other hand, decreased in all hydrogenated oils compared with the control.

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FLUID MIXING EFFECTS IN HYDROGENATION. James Y. Oldshue, Mixing Equipment Co., Inc., 135 Mt. Read Boulevard, Rochester, NY 14611.

The effect of fluid mixing on hydrogenation depends on 2 primary process steps. The first is the mass transfer of hydrogen to the liquid, which is a gas-liquid mass-transfer step. The second is the complex reaction with hydrogen dissolved in liquid and the reactants in the presence of solid catalysts. Gas-liquid mass transfer involves 2 different mechanisms in an impeller mixed system. If the hydrogen is added beneath the surface, the dispersion and mass transfer by the submerged impeller is an important part of the process. In many cases, a dead-end system is used and means must be provided at the upper gas-liquid interface to reincorporate hydrogen gas back into the liquid for further mass transfer. The mass transfer mechanism and scale-up correlations are different for a submerged mixing impeller and a surface reincorporation mixing impeller, and the separation of these 2 effects is necessary for proper scale-up or scale-down. Surface reincorporation phenomenon depends on whether tank baffles extend through the surface or not, and different correlating relationships will be presented. In terms of the chemical reaction, the 2 different concepts of macroscale/microscale mixing in fluid shear rates are involved. Techniques that determine the importance of each of these in the process are important for the proper analysis of laboratory and plant data. Scale-up involves the consideration of the mass-transfer steps and the chemical reaction mechanism, and also a consideration of various types of geometries to be used. Change in blend time and fluid shear rate distribution on scale-up is necessary for proper evaluation. An alternate or an adjunct to impeller mix tanks are side-arm circulators or complete pump-loop systems. Some of the pertinent characteristics of these various alternatives will be discussed.

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HYDROGENATION OF CANOLA OIL WITH A COMMERCIAL NICKEL CATALYST. L. deMan and J. M. deMan, Department of Food Science, University of Guelph, Guelph, Ontario, Canada, and R. G. Ackman, Canadian Institute of Fisheries Technology, Halifax, Nova Scotia, Canada.

Refined and bleached Canola oil was hydrogenated with Pricat 9906 catalyst to iodine values (IV) of 82 and 65 using various temperatures, pressures and catalyst concentrations. Hydrogenation with this catalyst resulted in great differences in SFI curves of fats with the same IV. Hydrogenation rate, dropping point, trisaturate content and linoleate selectivity were determined. The hydrogenated oils of IV 65 contained almost the same amount of solid fat at 20 C. For this reason, the crystal structure of the fats was examined by X-ray diffraction at 20 C. The catalyst was found to be nonselective. Increased selectivity can be obtained by careful tem-

perature and pressure control. Reactivity is also dependent on temperature and pressure. A high trisaturate content increased instability by promoting the formation of β crystals. The catalyst had excellent filtering characteristics.

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CRYSTAL STRUCTURE OF HYDROGENATED CANOLA OIL. Azza Naguib-Mostafa, A. K. Smith and J. M. deMan, Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Scanning electron microscopy was used to study the crystal structure of hydrogenated Canola oils. Fixation of the samples was carried out at 15 C using OsO_4 vapor in a sealed container. A standard system was first used—tristearin prepared in its 3 reported polymorphic forms: α, β', β. Micrographs were obtained for these forms showing the morphology of the crystals. A Canola sample that was selectively hydrogenated to an iodine value (IV) of 70 was detergent fractionated, washed, dried, and subjected to the same fixation procedure. Micrographs were obtained that indicate the predominant presence of the β-polymorphic form.

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HYDROGENATION OF CANOLA OIL USING RUTHENIUM CATALYST. Carmine Bello, Levente L. Diosady, W. F. Graydon and Leon J. Rubin, Department of Chemical Engineering, University of Toronto, 200 College St., Toronto, Ontario, Canada M5S 1A4.

Canola oil was hydrogenated using dichlorodicarbonylbis (triphenylphosphine) ruthenium (II) as a homogenous catalyst. The effect of temperature, pressure and catalyst concentration on reaction time, *trans* isomer formation and product distribution was compared with those obtained using commercial nickel catalysts (Nysel Hk-4, Unichemia Pricat 9906, AOCS standard catalyst). The ruthenium catalyst was active in the range of 100–200 C, 345 kPa (50 psig) to 5.17 MPa (750 psig) and catalyst concentration of 10^{-2} to 10^{-5} mol/kg oil. At 110 C, 5.17 MPa and 8.86×10^{-5} mol/kg oil, the observed reaction rate was 0.54 IV/min resulting in a maximum *trans* isomer content of only 9.2% at IV = 61.8. In addition, an iodine value drop of 40 units produced an oil containing 28% saturates, 60% monoenes, 11% dienes and less than 1% trienes. Product distributions were comparable to those produced by nickel. This catalyst is superior to the commercial nickel catalysts studied in terms of its ability to maximize activity and minimize *trans* isomer formation. Economic viability of this catalyst system depends on the recovery and reuse of the catalyst.

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CATALYTIC DECOMPOSITION OF METHANOL. Arne Kristiansen, Haldor Topsøe A/S Nymøllevej 55, 2800 Lyngby, Denmark.

A reliable source of cheap hydrogen in relatively small quantities is of great importance to many industries, particularly the natural oil and fat industry. Hydrogen production plants based on methanol decomposition constitute such a source and represent additional advantages, i.e., a very high degree of flexibility, fully automatic operation, short and automatic start-up and shut-down procedures, hydrogen purity according to requirements, design for outdoor erection, minimum site erection because of preerected design. By means of simplified process flow sheets, the paper describes the main process steps, which are: catalytic decomposition of methanol and simultaneous shift reaction; gas cooling and condensation and recycle of unreacted methanol; separation and purification of hydrogen by conventional processes or by pressure-swing adsorption (PSA); generation and transfer of the necessary heat for the reaction. Inherent merits of the process, including overall energy efficiency and economics, are discussed. The paper contains examples of consumption figures and investment cost.

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HOMOGENEOUS HYDROGENATION OF SOYBEAN OIL. S. Goldstein, I. Rave and A. Dolev, Koor Foods Ltd., P.O. Box 1597, Haifa, Israel.

Soybean oil was hydrogenated homogeneously using the catalyst suspended in an organic solvent. A series of hydrogenations were performed varying processing conditions and oil quality. The analysis of variance showed that the catalyst does not influence selectivity and *trans*-isomer formation compared with heterogeneous catalysis. The important advantage is the possibility to hydrogenate degummed oil and proceed to the refining stage. Hydrogenation with homogeneous catalyst may offer economic advantages over heterogeneous hydrogenation.

Session H Environmental Roundtable Monday p.m.

No abstracts.

Session I Physical/Chemical Basis of Protein Chemistry Monday p.m.

36

CURRENT ASPECTS OF SOYBEAN-PROTEIN FRACTIONATION AND NOMENCLATURE. J. R. Brooks and C. V. Moor, Clemson University, Clemson, SC 29631.

Soybean storage proteins consist of 3 major fractions, e.g., 7S and 11S globulins and whey proteins. The 7S and 11S globulins each contain multiple components and subunits that undergo association-dissociation reactions as a function of pH, ionic strength, dissociating agents and heating. These reactions drastically alter the physicochemical and functional properties of the soy proteins for use in food-product applications. Catsimpoolas and Ekenstam (1969) first reported the presence of multiple electrophoretic protein components in isolated β-conglycinin, the major component of 7S soy protein. They attributed this phenonemon to an association-dissociation reaction rather than to the existence of multiple protein isomers of the 7S protein. Thanh and Shibasaki (1977) and others have subsequently devised new and sophisticated procedures that provide improved fractionation and characterization of the 7S and 11S proteins and their components and subunits. However, numerous instances can be found where these reports disagree on the number of protein components and subunits, and on their molecular weight and physicochemical properties. These and other matters related to the current soy-protein nomenclature will be discussed and critically evaluated. In addition, the possible role of phytate in the above soy-protein fractionation and characterization will be considered. Also, efforts to fractionate soy proteins under high ionic strength, by gel filtration chromatography and gel electrophoresis, to minimize the association of 7S and formation of the 11S half-molecule will be presented.

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THE STRUCTURE AND COMPLEXITY OF 11S POLYPEPTIDES IN SOYBEANS. N. C. Nielsen, Purdue University, Department of Agronomy, West Lafayette, IN 47907.

The 11S soybean proteins called glycinin are isolated as a 350,000 dalton complex that consists of 6 nonidentical subunits. Each subunit consists of an acidic polypeptide component linked to a basic component by a single disulfide bond. Initial translation products of glycinin subunits are single polypeptides of ca. 60,000 daltons and undergo both co- and posttranslational modification. The precursors have a short signal sequence, followed by the acidic component, a short linker polypeptide, the basic component and a short trailer pesticide. Five major subunit types have been purified and characterized by amino-acid sequence analysis. While all of them are clearly synthesized by a family of homologous genes, they can be separated into 2 groups based on sequence homologies. Group I subunits ($A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}) are uniform in size (M_r = 58,000), relatively rich in methionine, and exhibit ca. 90% sequence homology among members in the group. The Group II subunits (A_3B_4, $A_5A_4B_3$) exhibit a similar level of homology among themselves, although they contain less methionine and are larger ($M_r \cong 62$–$67{,}000$) than Group I subunits. Sequence homo-

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logy between a member of 1 group and a member of the other is only 50–60%. Since the sulfur amino-acid content of subunits is variable and genetic polymorphism in subunit composition has been documented, an opportunity to alter the functional and nutritional properties of these seed proteins by genetic manipulation may be possible.

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BIOSYNTHESIS AND PROCESSING OF SOYBEAN 7S SEED STORAGE PROTEIN. R. N. Beachy, J. L. Bryant, M. A. Schuler, J. J. Doyle and D. Goeddette, Department of Biology, Washington State University, St. Louis, MO 63130.

The storage proteins in soybean (*Glycine max*) seeds accumulate in a developmentally regulated manner. The 2 major seed storage proteins, glycinin and β-conglycinin, accumulate in seeds as a result of the expression of a number of developmentally regulated genes. Our research on the β-conglycinins has included the isolation and characterization of gene sequences encoding both major and minor subunit components of this trimeric seed protein. The complete amino acid sequence of α′ and α subunits, derived from DNA sequences, were compared, assisted by computer programs, with each other and to the β-subunit of phasealin, the seed protein of *Phaseolus vulgaris* (garden bean) under the supposition that structurally important features of these proteins would be conserved throughout the evolution of these 2 plants. Our analyses showed that signal peptides of these proteins were highly conserved. Other regions were not highly conserved in amino acid sequence; however, predicted secondary structures of the protein were very similar. To extend our understanding of the structure, packaging, and breakdown of the β-conglycinins we prepared monoclonal antibodies and have identified a number of antibodies with a variety of unique and common features. The results of experiments monitoring the appearance of antigenic determinants during embryogenesis, and their disappearance during protein hydrolysis during germination will be discussed.

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EVALUATION OF SOME PHYSICOCHEMICAL PROPERTIES OF CHEMICALLY MODIFIED SOY GLYCININ. Wayne E. Marshall, James E. Sanderson and Susan M. Gaud, Kraft Research and Development, 801 Waukegan Rd., Glenview, IL 60025.

The use of soy protein in food has expanded considerably in the last 2 decades. However, the impact of soy protein in the cheese industry has been minimal since its use as a milk protein replacement causes color, flavor, and texture problems in cheese. In order to produce soy protein that will function like milk protein in cheese, information is required to determine the physicochemical properties of soy protein that affect cheese texture. Our presentation lays the groundwork for such an approach by identifying protein-specific physicochemical properties that may be involved in cheese texture, altering these specific properties by chemical modification and quantitating these properties as a prelude to evaluating modified proteins in cheese. The physicochemical properties chosen were solubility, molecular weight, surface hydrophobicity and protein structure. Soy glycinin was selected as our model protein. Selective chemical modifications, which include amidation, succinylation, or reduction followed by alkylation, were chosen to provide a broad range of physicochemical values. Modified proteins were characterized in a medium designed to simulate the low pH, high ionic strength conditions found in cheese. Modified glycinin solubility, molecular weight, and surface hydrophobicity varied according to the particular modification employed. All modifications altered glycinin structure from a molecule containing considerable β-structure to one that was mostly random coil. The results will be compared with similar data on sodium caseinate and discussed in terms of how these physicochemical changes may improve soy-protein function in cheese.

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GELATION OF SOY PROTEINS. Anne-Marie Hermansson, SIK—The Swedish Food Institute, Box 5401, S-402 29 Göteborg, Sweden.

An important functional property of soy proteins is the ability to form gels when heated. The gel network contributes to the characteristic texture of a food product and acts as a matrix holding water as well as other components. Denaturation and aggregation are the main reactions involved in protein gelation and the kinetics of these 2 reactions determine the type of structure formed. Both soy proteins—glycinin and conglycinin—have complex quarternary structures that easily undergo association-dissociation reactions. Glycinin has been shown to dissociate to subunits when dilute solutions (<1.0%) are heated. Mori et al. have shown that this may not happen at higher concentrations (5%). Results from a microstructure evaluation of glycinin and conglycinin gels will be presented in this paper. The monomer building up the glycinin gel strand has been found to have a diameter similar to that of the undissociated native molecule. This result can either mean that glycinin do not dissociate when heated in concentrated solutions and that undissociated but denatured molecules form the gel strands or that dissociation and association of subunits takes place before strand formation. In any case, the monomers of glycinin gel strands clearly consist of several and not of single subunits. Comparisons of the microstructure of glycinin and conglycinin gels show that the strands of conglycinin are branched with more loose ends than the strands of glycinin, which are more uniform. Depending on the temperature, the addition of sodium chloride can dramatically influence the microstructure of glycinin but not that of conglycinin gels. Commercially produced soy-protein isolates may behave quite differently than native soy proteins because of processing conditions that cause denaturation and various states of aggregation. Some examples of the functional properties and the microstructure of commercial soy-protein isolates will be given.

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CHARACTERIZATION OF THE FILM AND FOAMING PROPERTIES OF SOY PROTEIN FRACTIONS. J. B. German, S. H. Kim, T. O'Neill and J. E. Kinsella, Cornell University, Department of Food Science, 114 Stocking Hall, Ithaca, NY 14853.

The functional properties of soy proteins are dramatically affected by the forces stabilizing the native polypeptides and the interactions that develop as the native structure is altered in response to processing. Molecular flexibility is important in certain applications, e.g., foams. The foaming behavior of soy proteins was studied to examine how the structure of these proteins affected their surface and colloidal properties. Soy proteins were fractionated into 7S and 11S globulins by selective precipitation. The 11S globulin was then further purified into constituent acidic and basic subunits by ion exchange chromatography of the dissociated oligomers. Foaming behavior was monitored by bubble-size distribution and the kinetics of drainage. The surface rheology and film properties of the proteins were measured by the kinetics and force associated with the formation, thinning and collapse of single lamellae (tensiolaminometry). The ease of formation of foams and lamellae was correlated with the surfactancy or relative hydrophobicity of the proteins. Increasing the net charge on the proteins by changing the pH also lowered the foam strength and the viscosity of isolated lamellae. This effect was reduced by the addition of salt as a counterion. The stability of the foams was related to ability of the proteins to unfold and associate to form a viscous film. These observations will be discussed in the context of those molecular properties required for optimum foaming performance.

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EFFECTS OF HYDROPHOBICITY, CHARGE AND MOLECULAR STRUCTURE ON SOLUBILITY OF SOY PROTEINS. S. Nakai, S. Hayakawa and E. Li-Chan, University of British Columbia, 248 – 2357 Main Mall, Vancouver, B.C., Canada V6T 2A2.

Solubility and hydrophobicity, measured with *cis*-parinaric acid as a fluorescence probe, have been used for elucidating the functionality of various food proteins. However, protein solubility is a function of hydrophobicity and charge according to Bigelow (1967). Because using regressor variables that are strongly intercorrelated (multi-collinearity) in multiple regression analysis is not recommended, charge and hydrophobicity instead of solubility and hydrophobicity should be used in the regression analysis for

elucidating the protein functionality. The validity of Bigelow's hypothesis was investigated. The results indicated that the ANS hydrophobicity had better correlations than the parinaric hydrophobicity when used with charge (zeta-potential) as the regressor variables. The elution volume of proteins from a phenyl hydrophobic column replacing the ANS hydrophobicity further improved the correlation to their solubility. This result suggests that aromatic hydrophobicity may play a more important role than the aliphatic hydrophobicity in protein solubility. This phenomenon is similar to the specific adsorption of aromatic amino acids on cross-linked dextran gel from a mixture of amino acids, including aliphatic amino acids. According to Snyder (1978), the polarity scale that is an overall solvation capacity as well as the selectivity that is relevant to the chemical structure are essential for classifying the solvents. The π^* scale of Kamlet et al. (1977), another polarity scale, is usually greater for aromatic solvents than aliphatic solvents. This is ascribed to the higher polarizability of the aromatic solvents. The rigidity of molecular structure is also critical to the behavior of proteins relevant to their solubility. Facility in improving the solubility of soy protein by adding mercaptoethanol or by heating a weakly alkaline solution to temperatures higher than boiling point is suggestive of relatively loose folding of polypeptide chains in the protein molecules.

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MODIFICATION OF SURFACE CHARGES OF SOY PROTEIN BY PHOSPHOLIPIDS. Wen-Sherng Chen and William G. Soucie, Kraft Research and Development, 801 Waukegan Rd., Glenview, IL 60025.

Lecithin is used to prevent soy protein isolates from clumping in food processing. A System 3000 Electrokinetic Analyzer was used to investigate how the phospholipid modified the surface charge of soy protein. Electrophoretic mobility-pH curves showed that a crude commercial soy lecithin lowered the isoelectric point (pI) and increased the zeta (electrochemical) potential of the soy protein more profoundly than did a pure phosphatidylcholine. The modification of the surface-charge property, namely the pI and zeta potential, of the protein was a function of the phospholipid added. This result suggested that the commercial lecithin made the protein's surface more negatively charged and so dispersed it more readily in water than did the pure phosphatidylcholine.

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THE IONIC MODIFICATION OF THE SURFACE CHARGE AND ISOELECTRIC POINT OF SOY PROTEIN. William G. Soucie and Wen-Sherng Chen, Kraft Research and Development, 801 Waukegan Rd., Glenview, IL 60025.

The effect of anionic and cationic binding on the surface charge of soy proteins was measured by electrokinetic analysis. All of the ions investigated surpressed the surface charge of the protein; however, certain multivalent ions such as Al(III), Fe(III), hexametaphosphate and tripolyphosphate also altered the isoelectric point of the protein. The results indicate the unpredictability of ionic effects on protein functionality, emphasizing the importance of making direct measurements of protein surface charge.

Session J — Analyses by Iatroscan TLC/FID System — Monday p.m.

45

IATROSCAN OVERVIEW. John M. Newman, Newman-Howells Associates Ltd., Wolvesy Palace, Winchester, Hants SO23 9NB, England.

The history and development of the thin layer chromatography and flame ionization detection, a relative newcomer to conventional chromatographic techniques, will be presented, and the principles of separation and detection of the system will be described. In addition, applications of this instrument to various disciplines will be reviewed, with particular emphasis given to developments in Europe.

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A SPECIFIC DETECTOR FOR NITROGEN AND HALOGEN COMPOUNDS IN THIN LAYER CHROMATOGRAPHY (TLC) ON COATED QUARTZ RODS. P. L. Patterson, Detector Engineering & Technology Inc., 2212 Brampton Road, Walnut Creek, CA 94598.

A flame thermionic ionization detector (FTID) has been developed for use in the specific detection of nitrogen or halogen compounds present in samples analyzed by TLC on coated quartz rods. Similar to TLC/FID (flame ionization detector), a H_2-air flame is used to remove sample compounds from the coated quartz rods. The unique chemical environment of this flame converts samples containing nitrogen or halogen atoms into combustion products that are electronegative. These electronegative constituents in the flame effluent are selectively detected downstream of the flame by ionization on the surface of an electrically heated, ceramically coated, thermionic source. The method provides selective detection of nitrogen or halogen compounds even when those compounds are not well resolved chromatographically from other organic compounds.

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IMPROVED SAMPLE APPLICATION METHODS FOR THE IATROSCAN. Harry Read, British Petroleum Co. PLC, BP Research Center, ASRD, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN, England.

A new method of sample application has been developed for chromarods with the aim of improving interrod and interrun repeatability. The initial study involved comparing the recommended method of applying the sample by spotting with disposable micropipettes, and a static and rotary application using a micrometer-operated microsyringe. For the rotary method, a small rig was constructed to hold and rotate the rod as the sample was applied. This method gave improved interrod repeatability of 0.5—0.7% standard deviation compared with 1.3—3.3% standard deviation using the Iatroscan recommended method. Further work has resulted in a new improved frame designed to hold 10 Chromarods throughout an analysis (sample application, development and detection) without the need for any individual rod transfer operation. This rig has been constructed to allow the use of a proprietary aerosol applicator to give both improved sample application and concentration. Examples will be presented that will illustrate the development of this new method of sample loading using both the microsyringe and aerosol applicators.

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EFFECTS OF TEMPERATURE ON THE PHOSPHOLIPID COMPOSITION OF GILL TISSUE FROM THERMALLY ACCLIMATED RAINBOW TROUT (*Salmo gairdneri*). J. R. Hazel, Department of Zoology, Arizona State University, Tempe, AZ 85287.

The Iatroscan thin layer chromatography/flame ionization detector (TLC/FID) system was employed to follow the time course of changes in the phospholipid composition of gill membranes in rainbow trout following the transfer of 20 C-acclimated fish to 5 C and vice versa. Total lipid extracts were spotted on SII chromarods and initially developed in hexane/diethyl ether/formate (80:20:2). All of the chromarods were then scanned to within 1 cm of the origin and then subjected to a second development in chloroform/methanol/water (120:52.5/4.5). Following the second development the entire length of each chromarod was scanned. Equilibration of the chromarods at 52% relative humidity resulted in the best resolution. Linear standard curves were obtained for a variety of phosphatides (phosphatidylcholine, -ethanalamine, -serine, -inositol, sphingomyelin and lysophophatidylcholine) in the concentration range from 1—40 μgrams, however, the response factors differed 3—4 fold, depending on the phosphatide.

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CALIBRATION OF THE IATROSCAN-CHROMAROD SYSTEM FOR MARINE LIPID CLASS ANALYSES. C. C. Parrish, Department of Oceanography, Dalhousie University, Halifax, N.S. B3H 4J1, Canada, and R. G. Ackman, Canadian Institute of Fisheries

Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax, N.S. B3J 2X4, Canada.

A 2-step development procedure with partial scanning after the first development resolves seawater lipids into 7 classes. The low concentration in seawater of some of these classes necessitates calibration close to the detection limit of the flame ionization detector (FID). From 0.2 to 5.0 μg, the FID response is usually curvilinear, tending towards 0-0, but interrod precision is poor for most of this range. In an investigation of factors affecting FID responses, a relationship was found between the distribution of lipid material on a Chromarod as it enters the flame and the FID response for that material. Also investigated were the effects of temperature, humidity, acid cleaning, and double developments. All of these factors were found to have some effect on R_f values, on absolute FID responses and on reproducibility. Unless rods had been used many times without cleaning, and unless laboratory temperature and humidity varied extensively, these factors were not the major cause of variability in the Iatroscan-Chromarod system. However, the use of constant humidity chambers, daily cleaning of Chromarods, and development at a fixed temperature, is recommended. Similarly, double developments improve peak shape and response, but result in only a small increase in precision for some compounds. The differences among the FID responses obtained from 10 rods in a set imply that the normalization of FID responses to that of an internal standard, or the use of intrarod rather than interrod data, should result in an increase in reproducibility. Neither of these approaches was found to improve the precision for all lipid classes. An understanding of both interrod and intrarod variability, and the use of calibration curves, is essential for the accurate determination of the lipid classes of 1 L of seawater.

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LIPOPROTEIN LIPID QUANTITATION BY IATROSCAN. Roger D. Knapp and Bette C. Sherrill, Department of Medicine, M/S A-601, Baylor College of Medicine, 6565 Fannin, Houston, TX 77030.

An Iatroscan TH-10 was interfaced to an Apple II+ microcomputer with a Spectrum 1021 instrumentation amplifier/filter and an IMI A/D converter. The signal from the flame ionization detector was so noisy that heavy filtering was required before A/D conversion. No modification to the Iatroscan was necessary and no strip chart recorder was attached. As the data is acquired, the spectrum is displayed on the video screen. Ca. 300 data points are digitized for each of the Chromarods and stored on floppy disk as Applesoft text files. Software is in Applesoft BASIC, organized by modular function, to acquire and store data, retrieve data and calculate integrals, percentages and peak positions. Using this particular procedure, the data may be recalled, at the user's convenience, and manipulated by a variety of data-reduction programs and plot routines. In animal studies, this Iatroscan TH-10-Apple II+ microcomputer system has been used successfully to quantitate the changes in plasma lipoprotein lipids (cholesterol, cholesteryl esters, triglycerides, fatty acids and phospholipids) as a function of dietary changes.

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A COMPARATIVE EVALUATION OF THE ALCOHOL INDUCED FATTY LIVER BY THE TLC/FID AND GLC METHODS. G. Ananda Rao, Diana E. Riley, R. Thomas Crane and Edward C. Larkin, VA Medical Center (151H), 150 Muir Road, Martinez, CA 94553.

We quantitated alcohol-induced fatty liver by analyzing the triglyceride (TG) fatty acid methyl esters by gas liquid chromatography (GLC), using methylpentadecanoate as an internal standard, and compared the TG content obtained with flame ionization detector (TLC/FID). To obtain standard curves, various amounts of tripalmitin (TP) were spotted on chromarods and scanned with or without developing in benzene. The responses observed without developing rods were greater than those with developing rods. This result was also true for other compounds, such as phosphatidylcholine (PC), cholesterol and methyl palmitate. When small amounts of TP were spotted (2–4 μg), the responses of undeveloped spots were ca. 2-fold greater than developed spots. Proper conversion factors (μg/area) were used, depending on the amount of TG spotted. The extent of fatty liver observed by TLC/FID was ca. 2-fold greater than that by GLC when the amount of liver lipid spotted on the chromarods was small (2–8 μg TG). The TG contents, determined by the 2 methods, approached similar values when higher amounts of liver lipids were analyzed by TLC/FID. Liver cholesterol content, analyzed by the o-pthalaldehyde and TLC/FID methods gave similar values. When hepatic phospholipid content was ascertained using PC as a standard, values obtained by GLC were ca. 2-fold greater than those by TLC/FID. Although TLC/FID is advantageous for rapid multiple analysis of mixtures, its use for the quantitation of the components requires further understanding of the system.

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EVALUATING THE QUALITATIVE AND QUANTITATIVE ASPECTS OF THE IATROSCAN SYSTEM AND COMPARING IT TO OTHER METHODS. John K. G. Kramer, E. R. Farnworth, Animal Research Centre, Agriculture Canada, Research Branch, Ottawa, Ontario K1A 0C6, Canada, and B. K. Thompson, Engineering and Statistical Research Institute.

In the past few years, the Iatroscan has been evaluated for qualitative separations of lipid mixtures as well as quantitation of the resolved peaks. The purpose of this study was first to achieve maximum resolution of all the lipid classes as found in cardiac tissues. The solvent systems found to be most appropriate for the separation of neutral lipids were a combination of hexane/diethyl ether/formic acid (95:5:1) and 1,2-dichloroethane/chloroform/formic acid (92:8:0.1), run consecutively. The phospholipids were resolved using the solvent system: chloroform/methanol/water (68.5:29:2.5). Second, the study was undertaken to quantitate the lipid mixture and compare these results with those obtained from gas liquid chromatographic and chemical techniques. A comparison of the precision, accuracy, speed of obtaining the results and limitation of each technique will be presented and discussed. In addition, a method will be described to improve the results from the Iatroscan sufficiently to be of value in treatment comparisons.

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THE USE OF THE IATROSCAN TH-10 ANALYZER TO QUANTIFY TOTAL LIPIDS IN A VARIETY OF SAMPLE TYPES AND CHOLESTEROL, TOTAL BILE SALTS AND TOTAL PHOSPHOLIPIDS IN HUMAN GALLBLADDER BILE. H. Rodger Harvey, M. W. Rigler and J. S. Patton, Department of Microbiology and Institute of Ecology, University of Georgia, Athens, GA 30602.

A method for the measurement of total lipid weight in biological and geological lipid samples using the Iatroscan TH-10 analyzer is described. The method involves the application of small (5 μl) volumes to Chromorods, the focusing of the sample at 1 point by partial development in chloroform-methanol (1:1) or methanol, and the quantification by flame ionization detection (FID). The small response variation between different sample types did not affect the linearity of the response. The method exhibited a reproducibility of ±10% of the mean or better for samples ranging from 0.5 to 32.0 μg. The method, at least as sensitive and precise as microgravimetric procedures for total lipid determinations, allows total lipid measurement of 10 samples in 30 min. Using a double-development solvent system, the 3 major lipid components of bile—cholesterol, bile salts and phospholipids can be simultaneously analyzed on Chromorods. Ten samples could be quantified in less than 1 hr. The amount of bile added per rod was between 0.045 and 0.025 μl. The present techniques produced a low degree of variation/component/sample over a wide range of clinical and standard concentrations. Current methodologies for the analysis of bile lipids use a series of time-consuming assays. Quantification of bile-lipid classes by thin layer chromatography (TLC)-FID on Chromorods greatly simplifies the analyses of this important hepatic secretion.

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QUANTITATIVE CLASS SEPARATION OF COAL LIQUIDS USING THIN LAYER CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION. M. L. Selucky, Coal Research Department, Alberta Research Council, 11315 – 87th Avenue, Edmonton, Alberta T6G 2C2, Canada.

Class analysis of soluble liquefaction or pyrolysis products, based

on the precipitation of preasphaltenes and asphaltenes followed by column chromatography is lengthy and labor intensive and is not suited for treating a large number of samples. Thin layer chromatography using silica-coated rods and flame ionization detection as introduced by us removes most of the disadvantages of column and precipitation methods. Because, by definition, preasphaltenes and asphaltenes are solubility classes, chromatographic conditions have been developed based essentially on solubility, and moving oils to R_F ca. .9, asphaltenes to the middle of the bed, while preasphaltenes remain on the start. In the work with heteroatom-containing compounds, absolute detector calibration is mandatory. We will show that the response, which generally best fits regression of the type $y = ax^b$, where b lies between .8 and 1.2, can be substituted by a linear dependence over the range from 10 to 40 μg. Attempts at absolute calibration, complicated by solubility problems, have confirmed that unit detector response differences for the various classes are caused by differences in behavior of their solutions of varying concentrations rather than by substantial differences caused by the presence of heteroatoms.

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QUANTITATIVE DETERMINATION OF LIPIDS AND THEIR CONSTITUTIONAL MOIETIES BY CHROMAROD-TLC-FID SYSTEM. Toshihiro Itoh, Masamichi Tanaka and Hiroshi Kanoko, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 228, Japan.

The usefulness of a chromarod-TLC-FID system for the quantitative analysis of lipids has been already reported in our several papers, which include the argentation method and boric acid impregnated rod method. In this paper, we would like to present the application of this system for the quantitative analysis of constitutional moieties of archaebacterial lipids. The standard mixture of methyl glycoside, fatty acid methyl ester, and diglyceryl-dialkyl-tetraether were resolved on a chromarod S-II by a double developing technique. The components separated on the rod were automatically scanned with a hydrogen flame ionization detector (FID). Relative responses of methyl glucoside and methyl galactoside to fatty acid methyl ester and diglyceryl-dialkyl-tetraether were in the same proportions as the molecular ratio. This system was applied to the constitutional analysis of archaebacterial (sulfolobus) complex lipids. The molecular ratio of diglyceryl-dialkyl-tetraether and methyl hexoside in the methanolyzate of that bacterial neutral glycolipid (GL-2) was 1:1.9, which was very close to the theoretical value. Thus the thin layer chromatography (TLC) FID method was found to be quite useful for determining the relative amounts of complex lipid constituents.

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QUANTITATIVE ANALYSES OF TRIGLYCERIDES AND GEOMETRICAL FATTY ACID ISOMERS BY THE IATROSCAN TLC-FID TECHNIQUE ON $AgNO_3$ RODS. J. L. Sebedio, I.N.R.A.– Station de Recherches sur la Qualité des Aliments de l'Homme – 17 rue Sully – 21034 Dijon-Cedex, France, and T. E. Farquharson and R. G. Ackman, Canada Institute of Fisheries Technology, Canada.

The FID responses for the Iatroscan analysis on $AgNO_3$ rods of the *cis* and *trans* isomers of methyl esters of 18:1Δ6, 18:1Δ9 and 18:1Δ11 were studied at concentrations ranging from 0.5 to 20 μg using methyl stearate as internal standard. Generally, the FID correction factor was greater for the *cis* than for the *trans* isomer. Furthermore, 2 separate regressions, 1 for low sample loads, for example from 0.5 to 5 μg and 1 for the higher sample loads gave better correlation coefficients than a single regression using all the points from 0.5 to 20 μg. Very good correlation coefficients were also obtained using parabolic regressions. A separation of tristearin, triolein, trilinolein and trilinolenin was also obtained on chromatods-S impregnated with silver nitrate using a mixture of benzene, chloroform and acetic acid (90:8:2) as the solvent system. The FID responses for the triolein, trilinolein and trilinolenin were determined at concentrations ranging from 0.5 to 14.3 μg using tristearin as internal standard. Similar observations as described for the unsaturated methyl esters were reported for the triglycerides.

Session K Applications of Biotechnology Monday p.m.

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THE ROLE OF NITROGEN IN A MULTIORGANISM STRATEGY FOR BIOSURFACTANT PRODUCTION. N. Kosaric, W. L. Cairns, N. C. C. Gray, D. Stechey and J. Wood, The University of Western Ontario, Faculty of Engineering Science, London, Ontario N6A 5B9, Canada.

In an effort to improve the yields and costs for the production of microbial biosurfactants that can be used in enhanced oil recovery or other industrial applications, a multiorganism strategy is proposed. The strategy employs algae plus bacteria or yeast to first synthesize glycerides from waste feedstocks, and then a second organism (e.g., yeasts) to convert the glycerides to biosurfactants (e.g., glycolipids). The regulatory role of nitrogen is described for intracellular lipid accumulation by yeast, algae and bacteria and for extracellular biosurfactant production by yeasts. The implications of the role of nitrogen in lipid production are discussed in terms of implementing the multiorganism strategy of microbial biosurfactant production.

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THE BIOMODIFICATION OF FATS AND OILS WITH YEAST. Nabil Bati and Earl G. Hammond, Iowa State University, Department of Food Technology, Ames, IA 50011.

Many organisms modify the fats in their diet before depositing the modified fat in their own depots. We wished to see if finding yeast that could grow on triglycerides was possible and also accumulate significant amounts of oil as reserve energy supplies. Such yeast might provide an economically feasible way to upgrade inexpensive or inedible fats and oils to a product with more attractive properties. We tested a number of yeasts for their ability to live on triglyceride oil as a carbon source and also accumulate microscopically observable oil droplets. Of the fungi tested, the most successful seemed to be strains of *Candida lipolytica*. This organism also converts part of the oil it digests to citric acid and isocitric acid if given sufficient oxygen. To obtain maximum fat yield, the organism must be given plenty of oxygen during growth but the oxygen must be restricted after growth has reached its maximum. As opposed to yeasts that produce oil from carbohydrate, the deposition of oil by *C. lipolytica* was insensitive to nitrogen in the medium. Under the best batch culture conditions, 57% of the 27 g of oil in the medium was recovered as yeast oil and 11% was recovered from the medium. Also, 9.6 g/L of non-lipid yeast mass and 1.25 g/L of citrates were produced. The yeast-oil fatty acid composition tends to reflect the composition of the oil that is used as a carbon source, except that it contains more palmitoleic acid. Tocopherol in the carbon-source oil could not be recovered in the yeast but some of the sterols could. The yeast oil tended to have a high free fatty acid content.

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FURTHER ASPECTS OF WAX ESTER BIOSYNTHESIS BY *Acinetobacter* SP. H01-N. John Heigert and Saul L. Neidleman, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608.

Earlier reports from our laboratory have shown that *Acinetobacter* sp. H01-N can synthesize wax esters from diverse substrates: individual n-alkanes, acetic acid, propionic acid, ethanol and propanol. The chemical nature of the wax-ester mixture obtained is both substrate and temperature dependent. In a continuation of these studies, we have found that the use of n-alkane mixtures, including mineral seal oil, yields an even broader range of wax-ester mixtures than previously produced from single n-alkane substrates. In addition, 2 techniques for improving wax ester yields will be discussed: the use of mutant strains of *Acinetobacter* sp. H01-N and the presence of an excess of water-immiscible substrate, for example, n-hexadecane. In each case, we speculate that a reduction of wax-ester degradation contributes to the improved yields.

Meetings

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DEVELOPING A NEW INDUSTRIAL ENZYME APPLICATION-A STRATEGY. C. O'Donnell L. Boyce, C.O.L. Boyce, Novo Laboratories, Inc., P.O. Box D, Wilton, CT 06897.

Many companies view industrial enzymes as potential tools for making technological breakthroughs in their industries. Some have created special biotechnology groups to pursue this aim. Too often their projects are terminated before the intended goals are met. A major reason is too much time was consumed for too little perceived gain. A strategy is proposed to help R&D groups and their managers conserve valuable resources. It focuses on the essentials in evaluating new enzyme applications and in better developing discoveries. We will discuss the strategy's 3 main elements: (a) setting development criteria early on; (b) shortening learning curves and (c) using process monitoring techniques to ensure reproducibility batch to batch. Illustrative examples for a lipolytic and a proteolytic reaction are presented.

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ENZYMIC ESTERIFICATION AND INTERESTERIFICATION. R. Aneja, Cornell University, Section of Biochemistry, Molecular and Cell Biology, Clark Hall, Ithaca, NY 14853.

Enzyme technological approaches to the production of specialty lipids, including the triglycerides of cocoa butter, are the subject of intense current interest and studies. These use hydrolytic enzymes such as esterases, lipases and phospholipases as catalysts for esterification of acids with alcohols. Reactions are carried out with dispersions of lipid substrates in buffered enzyme solutions, or preferably in organic, low water content solvent systems. Substrate specificities inherent in the hydrolytic modes of the enzymes are largely retained in the esterification mode. With substrates comprising an ester mixed with an alcohol, or an acid, or another ester, reactions which are formally equivalent to alcoholysis, acidolysis or interesterification can be realized, these being the net results of initial hydrolysis and subsequent reesterification with new acid/alcohol partners. Mechanistically, the putative acyl-lipase intermediate is subject to competitive nucleophilic attack by an alcohol leading to an ester, and by carboxylate anion leading to a fatty acid anhydride. In line with this, fatty acid anhydrides, for example oleic anhydride, are substrates for lipases both in the hydrolytic mode and in the esterification mode. The discovery of this new class of substrates for lipases has implications for the kinetic description of the esterification reactions, the control of unesterified by-products and the development of novel routes to specialty lipids. Thus, the reaction of oleic anhydride with 1-palmitoyl-3-stearoyl *rac*-glycerol in the presence of the lipase from *Geotrichum candidum* gives 1-palmitoyl-2-oleoyl-3-stearoyl *rac*-glycerol, the major triglyceride of cocoa butter.

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PLANT-DERIVED CATALYSTS AND PRECURSORS FOR USE IN PROSTAGLANDIN SYNTHESIS. Tim J. Ahern, 16-210, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139.

Arachidonic acid derivatives such as prostaglandins, thromboxanes, leukotrienes and prostacyclin have been recognized to have significant pharmacological potential. They are not presently available commercially in large amounts because their low concentrations in living tissues and possession of multiple asymmetric carbon centers make the cost of their extraction or chemical synthesis prohibitively expensive. However, the theoretical feasibility of the commercial production of such C-20 compounds by means of fermentative and enzyme engineering processes that bypass animal sources entirely has been demonstrated. The precursor, arachidonic acid, is present in microorganisms adaptable to large-scale fermentation. For the purpose of this study, a model describing changes in arachidonic acid production by the red alga, *Porphyridium cruentum*, in response to induced lipogenesis and variation of light intensity and temperature was developed. In addition, the demonstration that immobilized microsomes containing prostaglandin synthetase activity in a stable amphiphilic gel eliminates the need for the expensive and time-consuming enzyme recovery methods used previously. Furthermore, the ability of a leguminous enzyme to catalyze the synthesis of prostaglandins indicates that cheap catalysts and precursors of plant origin are available for the synthesis of such compounds by the means described above.

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INDUSTRIAL-SCALE APPLICATION OF ENZYMES TO THE FATS AND OIL INDUSTRY. L. H. Posorske, Nova Laboratories, Inc., P.O. Box D, Wilton, CT 06897.

Commercial modification of starch and protein biopolymers by enzymatic means are well established as industrial processes. The use of enzymes to modify lipids is still largely in the developmental state. Lipolytic enzymes are being considered for production of fatty acids by total hydrolysis, as well as expanded use in the area of lipolysis for flavor modification. Nonlipolytic enzymes are being studied for their ability to improve extraction of edible oils. Examples of current research and applications development in these areas will be discussed.

Session L Fatty Acid Synthase: Structure, Function and Molecular Biology Monday p.m.

64

UROPYGIAL GLAND FATTY ACID SYNTHASE. P. E. Kolattukudy and A. J. Poulose, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

Uropygial gland, the main sebaceous gland of certain waterfowl, produces multiple methyl-branched fatty acids. The enzyme that catalyzes the synthesis of these acids from methylmalonyl-CoA was found to be identical to the synthase that generates n-fatty acids from malonyl-CoA in all its physico-chemical, catalytic and immunological properties. As this enzyme is the most abundant protein in the gland, it could be purified in large quantities by a simple gel-filtration step. This synthase is a dimer of 250 K dalton peptides. The monomers appear to be structurally and functionally identical as judged by the following criteria: (a) quantitation of the active sites by chemical modification showed that each monomer has 1 domain each of acyl carrier protein, β-ketoacyl synthase, β-ketoacyl reductase, enoyl reductase and thioesterase; (b) inhibition of the component activity of all the above catalytic domains by specific chemical modification correlated well with the inhibition of the overall fatty acid synthase activity; (c) specific chemical modification, followed by proteolytic digestion, yielded a single modified active site peptide from each of the following domains: acyl carrier protein, β-ketoacyl synthase, enoyl reductase and thioesterase domain; (d) amino acid sequence of the active site peptide fractions from these domains showed no heterogeneity. Intersite distance measurements, using fluorescence energy transfer between the chromophores bound at the acyl carrier protein and thioesterase domain, showed that the 2 sites are 50Å apart on the same peptide. In the multifunctional enzyme, interdomain interactions could play a regulatory role and enoyl reductase was suggested to play such a role based on the following observations: (a) NADP binding at the enoyl reductase domain resulted in the inhibition of β-ketoacyl synthesis, the rate-limiting reaction in the overall fatty acid synthesis; (b) NADPH binding at the enoyl reductase domain converts the catalytically inactive monomer into catalytically active dimer and protects the enzyme from proteolysis.

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EVOLUTION OF MAMMALIAN FATTY ACID SYNTHASE BY GENE FUSION. D. G. Hardie and A. D. McCarthy, Biochemistry Department, Dundee University, Medical Sciences Institute, Dundee, DD1 4HN, Scotland.

Mammalian fatty acid synthase is a remarkable multifunctional protein containing 6 active sites and an acyl carrier function on each of 2 identical polypeptide chains. In certain other organisms, such as

E. coli, all of these sites, including the acyl carrier function, are carried on distinct proteins. Recent studies suggest that the mammalian complex has been derived by the fusion of genes coded for separate proteins related to the present day E. coli system. We have been able to generate an active proteolytic fragment containing the acyl carrier of rabbit fatty acid synthase that is similar in size to, and shows extensive sequence homology with, E. coli and barley acyl carrier proteins. We will also present data showing that mammalian fatty acid synthase, unlike the yeast or E. coli systems, contains a single activity transferring both acetyl and malonyl groups from CoA to the acyl carrier, and that this activity may be related in sequence to the terminating thioesterase activity and also to the serine proteases. We have also mapped the positions of 5 of the 7 active centers of rabbit fatty acid synthase along the single, multifunctional polypeptide chain. Comparison of this map with the disposition of the activities between the 2 multifunctional polypeptide chains of yeast fatty acid synthase suggests that the mammalian and yeast systems may have arisen by independent gene fusion events.

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STRUCTURAL AND FUNCTIONAL MODE FOR FATTY ACID SYNTHETASE OF ANIMAL TISSUES. James K. Stoops and Salih J. Wakil, Biochemistry Department, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

The fatty acid synthetase of animal tissues consists of 2 subunits, each containing 7 catalytic centers and an acyl carrier site. Proteolytic cleavage patterns indicate that the subunit is arranged into 3 major domains: I, II, and III. Domain I has a molecular weight of 127,000 and contains the NH_2-terminal end of the polypeptide and the catalytic sites of β-ketoacyl synthetase (condensing enzyme) and the acetyl- and malonyl-transacylases. This domain functions as the site of entry and subsequent condensation of the acetyl and malonyl groups in the process of fatty acid synthesis. Domain II is the medial domain and contains the β-ketoacyl and enoyl reductases, the dehydratase and the 4'-phosphopantetheine binding site of the acyl carrier protein. Domain II, therefore, functions as the region where the keto group is reduced to the methylene carbon by sequential reactions of reduction, dehydration and reduction again. Throughout these processes, the acyl group is attached to the pantetheine-SH of the acyl carrier site, which is located within the 15,000 dalton polypeptide at the COOH-terminal end of Domain II and connects to Domain III. The latter domain contains the thioesterase activity, which is responsible for the release of palmitate from the synthetase subunit. Based on these results and the recent evidence that the synthetase subunits are arranged in a head-to-tail fashion, a functional model is proposed in which a palmitate-synthesizing center is comprised of Domain I of 1 subunit and Domains II and III of the second subunit. In this model, 2 sites for palmitate synthesis are constructed in the active synthetase dimer, a conclusion that we have verified experimentally.

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THE AVIAN FATTY ACID SYNTHASE GENE: MOLECULAR CLONING AND REGULATION OF mRNA SEQUENCE ABUNDANCE. Alan G. Goodridge, Robert A. Jenik, Michael A. McDevitt, John H. Nilson and Judith E. Fisch, Department of Pharmacology, Case Western Reserve University, 2119 Abington Road, Cleveland, OH 44106, and Sidney M. Morris, Jr., University of Pittsburgh, Pittsburgh, PA.

A double-stranded cDNA clone blank was constructed from total poly $(A)^+$ RNA from the goose uropygial gland. Clones containing sequences complementary to fatty acid synthase (FAS) mRNA were initially identified by colony hybridization with a ^{32}P-labelled cDNA transcribed from RNA enriched for FAS mRNA. Identity of the FAS clones was confirmed by hybrid-selected translation. Several different clones, representing about 3,500 bases of the mRNA, have been isolated. Mature FAS mRNA contains about 16 kilobases, more than twice the size required to code for the 250,000 Da subunit of the enzyme. Southern analysis of genomic DNA suggests that FAS is a single copy gene. In liver, FAS mRNA sequence abundance is very low before the hatching period, increases slowly during the hatching period and rapidly when newly hatched animals are fed. Starvation causes a rapid decrease in hepatic FAS mRNA. These changes correlate with the relative synthesis of FAS protein. The kinetics of the stimulation or inhibition of FAS mRNA sequence abundance, caused by feeding or starvation, suggest that the half-life of the mRNA is about 5 hr and is unaffected by starvation. In hepatocytes in culture in a chemically defined medium, triiodothyronine alone stimulates a 5-fold increase in FAS mRNA. Insulin alone has no effect, but in combination with triiodothyronine, a 12-fold increase in FAS mRNA occurs. Glucagon causes a 65% inhibition of the increase caused by triiodothyronine plus insulin. Effects of the hormones on enzyme activity and enzyme synthesis are virtually identical to those just described for mRNA except for triiodothyronine, which has little or no effect unless insulin is also present. Thus, triiodothyronine and glucagon regulate enzyme synthesis by controlling FAS mRNA sequence abundance whereas insulin does so by controlling both sequence abundance and translational efficiency.

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QUINARY INTERACTIONS OF LIPOGENIC ENZYMES. Paul A. Srere and Tracy C. Linn, VA Medical Center 151B, 4500 South Lancaster Road, Dallas, TX 75216.

Evidence is accumulating that the enzymes of several different metabolic pathways (pentose phosphate shunt, glycolysis, TCA cycle) exist as multienzyme complexes. We are examining the hypothesis that the enzymes of fatty acid synthesis, ATP citrate lyase (ATP-CL), acetyl CoA carboxylase (ACC) and fatty acid synthetase (FAS) also exist as a complex. Our initial efforts to test for a kinetic effect on the overall pathway by mixing the 3 pure enzymes in vitro led to the observation that FAS has an absolute requirement for free CoA. This specific effect has been further developed by Smith and coworkers. Other experiments to show physical association of ATP-CL and FAS by the use of molecular sieve chromatography or sucrose density centrifugation did not reveal any interactions. We next designed experiments to test the possibility that the presence of a cellular membrane component might facilitate the formation of a complex. Because we have previously shown a functional relationship of ATP-CL to mitochondria, we tested for the binding of this enzyme to mitochondria and found that some binding could be observed. The work of Janski and Cornell indicated that a considerable portion of ATP-CL did not behave as a free cytosolic enzyme and their studies indicated that the enzyme bound to mitochondria. However, as some evidence already existed that ACC was associated with the endoplasmic reticulum and because the product of FAS, palmitate, was used by an enzyme associated with endoplasmic reticulum, we then tested and successfully demonstrated that a significant amount of ATP-CL can bind to the microsomal fraction. Evidence indicates that ATP-CL (but not FAS) binds to endoplasmic reticulum to an extent that can easily account for the bound fraction reported by Janski and Cornell. This binding occurs to a protein component of that membrane and can be dramatically altered by physiological concentrations of CoA and ATP but not citrate. So far we have not observed any difference in the binding capacity that can be ascribed to the state of phosphorylation of ATP-CL.

Session M Plant Engineering—General Tuesday a.m.

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A COMMERCIAL EXPERIENCE OF CONDITIONING RAW SOY FLAKES AT ELEVATED TEMPERATURES PRIOR TO SOLVENT EXTRACTION. N. W. Myers, Myers Engineers, P.O. Box 1493, Decatur, IL 62525.

A commercial experience is described in which the Filtrex process was used in a solvent extraction plant for soybeans in Mexico. The process entailed preconditioning the soy flakes at 100 C and elevating the moisture level before extraction. The primary disadvantage encountered was the overtoasted effect on the nutritional qualities of the soy meal, which was used primarily in the broiler production industry in Mexico. The process was discon-

tinued and the extraction plant rebuilt using conventional pretreatment procedures. Plant experiences and modifications are described in detail. The final result of this experience is that the modified plant is presently producing high-quality soy meal at .25 (.1 to .5 range) urease activity (as measured by pH change) with an increased solvent loss compound with that of conventional plants.

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PRACTICAL RESULTS IN A NEW SUNFLOWER OIL PLANT: IMPROVEMENT OF OIL AND MEAL QUALITY. Z. Leibovitz and C. Ruckenstein.

A description of National Sun Industries, an oil plant in Enderlin, North Dakota, and the results after 18 months of crushing sunflower seed. Reception and storage of the seeds. The importance of drying before decortication after normal storage. Advanced decortication is used to obtain high-protein meal. Preparation and pressing of the seed. The improvement of oil quality gave low waxes content, good color and lower acidity. Cake preparation and extraction was done with an HLS/T.O.M. extractor. Special wet degumming produced oil with very low phosphorus content that was suitable for physical refining. The advantages of meal with 40–41% protein was compared with other sunflower meals. The advantages of building factories in the vicinity of the sunflower fields is discussed.

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THE PHYSICAL REFINING OF SUNFLOWER OIL. Anthony Athanassiadis, De Smet Engineering.

In recent years, the developments made in the physical refining of unsaturated oils have enabled some oil-millers to apply the process to sunflower oil. The main reasons behind the choice of physical refining in this case, as against caustic refining can be described as follows. The average free fatty acids (FFA) of crude sunflower oil is generally between 1% and 2%, which cannot be neglected. The economic interest of physical refining, therefore, becomes clearly marked: oil losses are reduced as the FFA are removed by distillation. In fact, the refining factor with the deacidification process by distillation is limited to 1.1, whereas with a conventional system, this factor exceeds 1.6. The quality of crude oils, a mixture of expelled oil and extracted oil, available in producing countries is fairly stable; on top of this, they always fulfil the criteria of physical refining. Hence, from a purely technological point of view, the decisive condition, i.e., the perfect purification of the oil before deacidification by distillation, is met within nearly all varieties of sunflower oil. A thorough study of degumming and pretreatment efficiency with data of industrial application are mentioned in the paper, including process diagrams. The 2 considerations mentioned above do not, however, close the question and potential users should not forget the determining advantages of physical refining in all cases, e.g., investment cost, pollution, processing cost. Some comparison between the processing cost of conventional and physical refining is given.

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PHYSICAL REFINING—A PUZZLEMENT. Calvin T. Zehnder, Cherry Burrell, P.O. Box 35600, Louisville, KY 40232-5600.

This paper will attempt to present consideration–evaluations–commitments which an edible oil refiner should review in detail in deciding the degree to which he pursues physical refining versus caustic refining in his operation. The actual process of physical refining will not be addressed since there have been many papers on this subject. Included will be such things as capital expenditure impact, existing versus new installation considerations, operating economies, product quality control particularly related to soybean oil, and handling of disposable by-products, i.e., soybean gums versus acid oil, bleaching earth, deodorizer distillates, etc.

73

WINTERIZATION OF SELECTIVELY HARDENED SOYBEAN OIL. A. Tirtiaux, Tirtiaux S.A. Fractionnement Tirtiaux-Fleurus, 601 Chaussée De Charleroi, B. 6220 Fleurus (Belgique).

With its high content of linolenic acid, soybean oil has poor stability and oxidizes easily. The developments of new catalysts for hydrogenation have partially solved this problem. With the use of these, combined with the special conditions of process operation, the linolenic acid (C 18:3) is selectively hardened into linoleic acid (C 18:2) rather than switching from linoleic (C 18:2) to oleic (C 18:1) and stearic (C 18:0) acids. As a result, the hardened product has an increased stability that increases even more as the iodine value decreases. On the other hand, the content of solids in the oil has reached higher values and the product fails to pass any form of cold test. The removal of these solids (winterization) is part of the second stage of the stabilizing operation. The author proposes, with the help of diagrams, slides and detailed analysis, to report on a comprehensive trial of combined hardening and winterization made by Tirtiaux on soybean oils selectively hydrogenated to iodine values of 107 to 91 and below. Additional data obtained on the Tirtiaux mobile pilot plant in the US will also be displayed, showing the excellent performances of the Tirtiaux fractionation process in the field of winterization of selectively hardened soybean oil.

74

CURRENT CAPITAL AND OPERATING COSTS FOR SOYBEAN OIL REFINERIES. J. Stephen Harris and Philip A. Bollheimer, PSI Process Systems, Inc., 4466 Elvis Presley Boulevard, Memphis, TN 38116.

Capital cost and operating cost estimates for a soybean-oil refinery will be presented. A soybean-oil refinery, using the basic processing steps of caustic refining, bleaching, hydrogenation and deodorization, will be used as the base case. Examples of a 15,000 and a 30,000 lb/hr refinery will be given. A process description detailing areas where operating costs can be reduced will be given. Capital costs will be based on detailed engineering studies and previous design and construction experience. Operating costs will include chemical costs and average labor and utility costs.

75

POSSIBILITIES FOR THE USE OF SPENT BLEACHING CLAY. Werner Zschau, Sued-Chemie Ag, Lenbachplatz 6, Munich, West Germany.

Spent bleaching clay is a by-product that, by most people, is regarded as an embarrassing material, just causing costs and headaches. For quite a long time, this has been the general feeling of most people involved with it. As a producer of bleaching clay, we have looked into that problem for the last 15 years, and this paper will show some of the possibilities we, as well as others, have developed. Special consideration is given to the aspect of energy saving.

Session N Lipid Nutrition—Health and Disease Tuesday a.m.

76

SERUM LIPIDS AND LIPOPROTEINS IN FASTED AND REFED PONIES. John E. Bauer, University of Florida, J-144 JHMHC, University of Florida, Gainesville, FL 32610.

Seven 2 year-old pony mares were fasted for a total of 160 hours and then refed. Four normally fed animals were used as controls. With fasting, serum triglycerides (TG) increased 24-fold, whereas cholesterol levels doubled. The relative amounts of prebeta lipoproteins (LP) increased from 6% to 23%. Concomitant decreases in the percentage of alpha LP were also observed. Compositional analysis of the $d < 1.006$ g/ml LP fraction (VLDL) revealed increases in the relative amounts of TG and cholesterol and a decrease in total protein. On refeeding, these parameters all returned or began to return to their normal levels. Postheparin plasma triacylglycerol hydrolase (PHP TGH) activities did not change with fasting. During refeeding, however, these activities increased significantly and subsequently returned to their normal prefasting values. Lipoprotein lipase (LPL) activities of individual ponies were purified via affinity chromatography and averaged 250 fold, with recoveries of 50% and

93%. The enzyme was more than 90% pure by SDS-PAGE with an apparent 66,000 MW. With fasting, no change in the specific activity of the purified LPL was observed but an increase was noted during refeeding. Although hyperlipemia in the fasted pony is variable, it nevertheless appears unique for this species. This response may reflect the ability of the pony to mobilize massive amounts of fatty acid with their subsequent reesterification in the liver. Changes in LP composition appear to be only minor in response to fasting. Of particular interest, were changes in the relative protein composition of the fasted pony VLDL with the appearance of large amounts of prebeta migrating LP on agarose gels. The cause of the hyperprebetalipoproteinemia does not appear to caused by decreased PHP TGH or purified PHP LPL specific activities. These findings, however, do not rule out increased hepatic (VLDL) synthesis or other causes of decreased VLDL catabolism as possible mechanisms. Similarities of this animal model with human type IV hyperlipoproteinemia will be discussed.

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MEDIUM CHAIN TRIGLYCERIDES (MCT) MAY BE THE OPTIMAL ENERGY SOURCE IN HEPATIC INSUFFICIENCY. James J. Pomposelli, Vigen K. Babayan and George L. Blackburn, Harvard Medical School, 194 Pilgrim Road, Boston, MA 02215.

Chronic hepatic disease is a life-threatening illness and is commonly associated with progressive malnutrition. Currently available long-chain triglyceride (LCT) lipid emulsions are frequently contraindicated. Because of the different metabolism of medium-chain triglycerides (MCT), we undertook a study to examine the efficacy of various total parenteral nutrition (TPN) formulas containing different nonprotein calorie sources in rats with experimentally induced hepatic insufficiency by portacaval anastomosis. All animals were randomized to receive 1 of the following TPN formulas for 3 days. The control group received 175 kcal/kg BW/day as 12.5 g amino acids/kg/day and dextrose. Groups 2, 3 and 4 received an additional 125 kcal/kg BW/day as either MCT/LCT (1:1), LCT or dextrose, respectively. Group 5 received 300 kcal/kg BW/day as dextrose only. Despite similar nitrogen balance among groups 1–4, serum albumin concentrations were highest in rats receiving MCT/LCT ($p<0.05$). Normal liver morphology was observed in the MCT/LCT group where glycogen and triglyceride deposition was seen in animals receiving LCT and dextrose only. Animals receiving only dextrose were in negative nitrogen balance and had significantly lower albumin synthetic rates ($p<0.05$). The results of this study support the conclusion that MCT as a component of TPN formulas is preferable to LCT or dextrose only as an energy source in hepatic insufficiency. These data contradict current dogma that suggests that lipids in general are not well tolerated in liver disease.

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THE ROLE OF MEDIUM CHAIN TRIGLYCERIDES (MCT) IN PRESERVING RETICULOENDOTHELIAL SYSTEM (RES) FUNCTION IN IMMUNOCOMPROMISED RATS. Karim Hamawy, Kazuma Yamazaki, Vigen Babayan, Anthony Valicente and George Blackburn, Harvard Medical School, N.E. Deaconess Hospital, 194 Pilgrim Road, Boston, MA 02215.

Maintaining immune system function is an essential requirement of patients suffering from hepatic insufficiency. The use of long-chain triglycerides (LCT) in immunocompromised patients is often contraindicated because of possible opsonin deficiency, reticuloendothelial system (RES) blockage and the hepatic triglyceride infiltration associated with such infusions. Because medium-chain triglycerides (MCT) are rapidly oxidized and not sequestered in the liver, we investigated the role of such lipid emulsions in the immunocompromised rat. Twenty-four male SD rats underwent portacaval anastomosis and splenectomy and were randomized to receive 1 of the following total parenteral nutrition (TPN) formulae for 5 days. Diets contained 200 kcal/kg BW/day as 8.8 g amino acid/kg BW/day, 24.5 g/kg BW/day as dextrose and 16.6 g/kg BW/day as either MCT (Group I) or LCT (Group II). Following TPN, a weight-related dose of ^{59}Fe-labelled *E. coli* was administered. The LCT group had a significantly higher number of bacteria remaining in the blood 30 min and 60 min after the administration of *E. coli* ($p<0.05$). Rats fed MCT had increased blood bacteria clearance, and sequestered fewer organisms in the lungs ($p<0.05$) and more in the liver ($p<0.01$). Thus RES function in the liver is reduced by LCT, whereas MCT feedings are associated with improved bacteriocidal capacity. These findings suggest that in the immunocompromised patient who needs intravenous nutritional support, MCT may play a major role in minimizing the potential for bacteremia and sequestration of organisms in the lung.

79

CHOLINERGIC STIMULATION OF PHOSPHATIDYLINOSITOL TURNOVER IN RETINOBLASTOMA CELLS. D. T. Dudley and A. A. Spector, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

We are investigating the agonist-induced turnover of phosphatidylinositol (PI), using the human retinoblastoma (Y-79) cell line. Treatment of cells for 10 min with acetylcholine stimulates the incorporation of ^{3}H-inositol into PI. After cells are prelabeled for 40 min with ^{32}P, stimulation with carbachol, an acetylcholine analogue, for 10 min results in increased labeling in PI and phosphatidic acid (PA), whereas labeling of phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylcholine (PC) is unchanged. Furthermore, atropine but not d-tubocurarine inhibits the cholinergic stimulation of PI labeling, suggesting the involvement of a muscarinic cholinergic receptor. Dopamine, norepinephrine and serotonin have no effect, whereas the calcium ionophore A23187 produces a large stimulation of PI labeling. This suggests involvement of calcium, as has been shown in other systems. The effect is rapid; increased PA labeling is seen by 1 min, and increased PI labeling by 3 min, suggesting a conversion of PA to PI. Incubation of Y-79 cells maintained in a serum-free medium with 1-^{14}C-arachidonic acid (20:4) shows a rapid incorporation of 20:4 into PI, with slower incorporation into PS, PE and PC. Additionally, chasing ^{14}C-20:4-labeled cells with unlabeled oleic acid (18:1), 20:4, eicosapentaenoic acid (20:5), or docosahexaenoic acid (22:6), shows that only 20:4 can cause a depletion of ^{14}C-20:4 from labeled PI. By contrast, all of the fatty acids can cause depletion of 20:4 from PS, PE and PC. This shows a specific tenacity of PI for 20:4. Work is currently under way to evaluate whether the 20:4 content of the PI fraction can influence cholinergic-stimulated PI turnover. These results show specific metabolic properties of PI that may be related to intercellular communication and signal transduction, and further show that the Y-79 retinoblastoma may be a good model for examining the interaction between muscarinic receptors and PI turnover.

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PLASMA TRIGLYCERIDE SECRETION AND PLASMA AND LIVER LIPID CONCENTRATIONS. COMPARISON OF RATS FED A FAT-FREE DIET OR DIETS CONTAINING 5% SATURATED FAT WITH RATS FED THESE DIETS PLUS 1% SAFFLOWER OIL. M. A. Williams, J. Tinoco, I. Hincenbergs and B. Thomas, Department of Nutritional Sciences, Room 119 Morgan Hall, University of California, Berkeley, Berkeley, CA 94720.

Metabolic responses of rats to lack or supplementation with essential fatty acids (EFA) may vary, depending on whether the basal diet is fat-free or contains saturated fat. Therefore, we have compared parameters of lipid metabolism in male rats fed EFA-deficient diets that are fat-free or contain 5% hydrogenated coconut oil (HCNO) or hydrogenated cottonseed oil (HCSO), as well as in controls fed these diets supplemented with 1% safflower oil (SAFF). These diets were fed ad libitum for 8–9 weeks after weaning. In fed rats, fractional rate constants (FRC) for plasma triglyceride (TG) secretion (Triton) and TG secretion rates (mg/min/100 g body weight) were greater, but plasma TG concentrations were lower in all deficient groups compared with their corresponding controls. Liver cholesteryl ester and TG concentrations were significantly higher in all deficient groups compared with their respective controls. Plasma cholesteryl esters were significantly lower in deficient rats fed the 5% HCSO diet, but not in other deficient groups. Liver cholesteryl ester concentrations were significantly higher in the 1% SAFF group than in the other EFA-supplemented groups. In rats fasted 15–16 hours, TG secretion rates were again greater in all de-

ficient rats than in their respective controls. FRC values were greater and plasma TG values lower in the 0 fat and 5% HCNO groups, but not in the 5% HCSO group.

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THE EFFECT OF PROPRANOLOL ON RABBIT ARTERIAL ACAT AND PLASMA LCAT, IN VITRO. F. P. Bell, The Upjohn Company, Kalamazoo, MI 49001.

Propranolol is commonly prescribed for the management of hypertension. Recent studies in man, however, have shown that the drug elevates plasma VLDL-triglycerides, elevates LDL-cholesterol and lowers HDL-cholesterol. Since the pattern of lipid/lipoprotein changes is considered to constitute a risk for the development of ischemic heart disease (atherosclerosis), concern exists that this risk could eventually offset the benefits of controlling blood pressure. Paradoxically, propranolol has been reported to have antiatherosclerotic effects in cholesterol-fed rabbits and roosters, which suggests that the drug may have direct effects on arterial metabolism that protect against atherosclerosis development despite the presence of risk factors. To investigate this possibility, lipid metabolism was studied in arterial tissue from cholesterol-fed rabbits in the presence of propranolol, in vitro. Propranolol was found to be an inhibitor of arterial microsomal ACAT (acylCoA:cholesterol acyltransferase), a key enzyme in the atherogenic process. The possible significance of these studies to the mechanism of the antiatherosclerotic effect of propranolol in experimental atherosclerosis will be discussed. Propranolol also inhibited rabbit plasma LCAT (lecithin:cholesterol acyltransferase), an enzyme involved in the normal metabolism of the plasma lipoproteins.

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FATTY ACID COMPOSITION OF THE MAJOR SERUM LIPID CLASSES IN PATIENTS WITH CYSTIC FIBROSIS AND IN THEIR PARENTS. Armand Christophe, Warren J. Warwick and Ralph T. Holman, The Hormel Institute, University of Minnesota, 801 – 16th Avenue, N.E., Austin, MN 55912.

The fatty acid compositions of the serum phospholipids (PL), triglycerides (TG) and cholesterol esters (CE) of 15 patients with cystic fibrosis (homozygotes) and 14 parents (heterozygotes) were determined by capillary gas chromatography. Both groups were compared with normal control groups. Both homozygotes and heterozygotes differed from normals in their linoleic acid metabolites. In the PL, 20:2ω6 was suppressed to 87% and 74%, 20:4ω6 to 80% and 76%, and 22:4ω6 to 35% and 28% of normal values, for homozygotes and for heterozygotes, respectively. Arachidonic acid was also suppressed in CE (78% and 72%) and in TG (75% and 79%). Homozygotes, but not heterozygotes, differed from normals in the linoleic acid content in their lipid fractions (82% of normal in the PL, 85% in CE, and 55% in TG). Four homozygotes had normal linoleic acid values in all lipid fractions studied, but these had as low values for linoleic acid metabolites as the rest of the group. These data support the theory of a defect in essential fatty acid metabolism in cystic fibrosis.

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EFFECT OF THE MODE OF ADMINISTRATION OF LINOLEIC ACID TO PATIENTS WITH CYSTIC FIBROSIS. Armand Christophe, Eddy Robberecht and Gaston Verdonk, Laboratory of Dietetics & Nutrition Research, University of Gent, Pasteurlaan 2, B 9000 Gent, Belgium.

Linoleic acid (LA) was administered to patients with cystic fibrosis (CF) as linoleic acid-rich monoglycerides (LAM), as mixtures of LAM with medium-chain triglycerides (MCT) and as a randomized corn oil/MCT mixture. The administration of LAM, which can be absorbed as micelles without prior digestion, and of LAM, to which MCT were added to improve solubilization of the former, caused marked increases in previously low LA values in the major lipid classes. These increases were accompanied by decreased monoenoic acids as would be expected in improvement of essential fatty acids status. However, arachidonic acid did not increase, indicating a fault in the metabolism of linoleic acid. After longer administration of these food fats, the reduced serum lipid and HLD levels rose moderately, probably as a result of improved fat absorption. Administration of the randomized mixture, which was expected to be a good substrate for lingual lipase in CF, had no effect on serum lipid levels or fatty acid composition. These results suggest that a more complete normalization of disturbed serum lipid fatty acid patterns of CF is to be expected when longer chain ω6 acids are administered as monoglycerides together with LAM.

84

HYPER- AND HYPO-RESPONSIVE INDIVIDUALS TO DIETARY CHOLESTEROL AMONG LABORATORY ANIMALS AND HUMANS. A. C. Beynen, Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, and L. F. M. van Zutphen, Department of Laboratory Animal Science, University of Utrecht, Yalelaan 1, 3508 TD Utrecht (The Netherlands).

We have observed 5–10 fold between strain differences in the response of serum cholesterol to cholesterol-rich diets in inbred strains of rabbits and rats. Therefore, differences in susceptibility to dietary cholesterol have a genetic basis. This finding was substantiated by the serum cholesterol responses observed in male rats from crosses between hyper- and hyporesponding inbred strains. We calculated that the response has a heritability greater than 80%, and that only a few major genes are involved. In man the response to cholesterol in the diet is variable, but in the absence of repeated experiments, the reproducibility of the response per person was not known, and the existence of human hyper- and hyporesponders remained uncertain. Therefore, we have carried out 3 controlled dietary trials, each lasting 4 to 8 weeks, and employing the same subjects. In each trial, the subjects successively consumed a low-cholesterol diet (about 120 mg cholesterol/day) and a high-cholesterol diet (about 650 mg cholesterol/day). Fifteen putative hyporesponders and 17 hyperresponders with mean serum cholesterol increases of −0.01 and 0.96 mmol/l (0 and 37 mg/dL) were selected in the first experiment and participated in the second and third trial. We found that the selected hyperresponders consistently displayed a significantly greater cholesterolemic response than the selected hyporesponders in the 2 further experiments, although the individual response was only partly reproducible. Thus, at least part of the serum cholesterol response to dietary cholesterol is individually determined in man. This finding was confirmed in another population. In 1976, in 34 subjects who habitually ate at least one egg a day, cessation of egg consumption, which caused a decrease in cholesterol intake of about 500 mg/day, was found to lower total serum cholesterol levels by 0.16 ± 0.42 mmol/l, with individual responses of −1.27 to +0.51 mmol/l. In 1982 the same subjects were reinvestigated and, on our request, they again eliminated eggs and egg-containing products from their diet. Significant, positive correlation ($r=0.32$, $p<0.05$) between the individual cholesterolemic responses in 1976 and 1982 was found. Thus, although the response in each volunteer was only partly reproducible from 1 study to another, we have presented evidence that human hyper- and hyporesponders do exist. Hyperresponsiveness to dietary cholesterol may be important as it could explain elevated serum cholesterol levels in subjects who have no clearly defined metabolic defect. The studies with humans were supported by the Netherlands Heart Foundation, grant 31.013 and an established investigatorship to M. B. Katan.

Session O Analysis of Waxes Tuesday a.m.

85

ANALYSIS OF WAX ESTERS BY GLASS CAPILLARY GAS CHROMATOGRAPHY. Stuart G. Wakeham and Nelson M. Frew, Department of Chemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Complex mixtures of wax esters and steryl esters from various natural samples are routinely analyzed by high-resolution and high-temperature glass capillary gas chromatography. These low-volatility neutral lipids, containing up to 60 carbons, are chromatographed on capillary columns deactivated and coated with 0.15 μm film of

cross-linked SE-52. With hydrogen as the carrier gas and an upper temperature limit of 370 C, columns of up to about 25 m in length may be used. Discrimination effects of conventional vaporizing injectors are reduced by the use of a nonvaporizing, on-column injector. This procedure may be extended to gas chromatography and mass spectrometry. A discussion of analytical conditions and the application of the method to the analysis of wax and steryl esters in marine samples will be presented.

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COMPOSITIONAL ANALYSIS OF NATURAL WAX ESTER MIXTURES BY TANDEM MASS SPECTROMETRY. Gayland F. Spencer and Ronald D. Plattner, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1815 N. University Street, Peoria, IL 61604.

Tandem mass spectrometry is particularly suited for the analysis of complex, natural wax ester mixtures $R_1-CO_2-R_2$. Reducing the mixture with deuterium provides species that are separable (through mass spectrometry) based on the number of original double bonds. Chemical ionization with isobutane produces high yields of protonated molecular ions and very little further fragmentation. These ions are separated by the first mass filter and then dissociated through collisions with argon. The positively charged dissociation products are almost exclusively the protonated acid ions $(R_1-CO_2H_2)^+$, which can then be separated by the second mass filter before detection and quantitation. The technique overcomes many of the obstacles previously faced during wax ester analysis. Results from this method are compared with those obtained by previous work.

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ANALYSIS OF WAXES FROM VERTEBRATE SKIN. Donald T. Downing, Mary Ellen Stewart, Philip W. Wertz and Sabin W. Colton VI, 270 Medlabs, University of Iowa, College of Medicine, Iowa City, IA 52242.

Profound interspecific variation in skin lipid composition provides exercises in lipid analysis and structure determination. In addition to a variety of wax esters, skin lipids include: ω-lactones (horse, donkey and zebra); squalene (man, otter, beaver and kinkajou); glycerol ether diesters (guinea pig); and free, odd-carbon fatty alcohols and methyl ketones (Indigo snake). Skin lipids often contain high proportions of methyl-branched chains and unusual double-bond positions. The polar lipids of mammalian skin contain 14 series of ceramides and glycosylceramides composed of sphingosine or phytosphingosine in amide linkage with fatty acids, α-hydroxyacids or ω-hydroxyacids, and esterified acids consisting predominantly of linoleic acid. Lipid class analyses are obtained routinely by quantitative thin layer chromatography (TLC) involving H_2SO_4-charring and photodensitometry. Preparative TLC allows for the isolation of most lipid classes, which are then subjected to diagnostic chemical dissection, followed by NMR , ultraviolet (UV) and (IR) spectrometry. The aliphatic fractions are separated into saturated, monoenoic and polyenoic series by $AgNO_3$ TLC before gas liquid chromatograph (GLC) analysis. The frequent presence of chain branching, in the form of 1 or more methyl side chains, requires the use of capillary GLC for adequate resolution and analysis, and of GLC and mass spectrometry (MS) for structure determination. In favorable cases, as in the iso-branched acids and hydroxyacids from horse sebum, structure determinations can be achieved unequivocally by NMR. For determination of positions of unsaturation in aliphatic chains, individual chain length fractions isolated by preparative GLC are subject to $NaIO_4/KMnO_4$ oxidation and GLC analysis of the fragments. Volatile fatty acids are handled as involatile tetramethylammonium salts, which pyrolyze to methyl esters at 300 C in the flash heater of the gas chromatograph. Only a limited number of organic reactions have proved suitable for lipid analysis because of the requirement for quantitative conversion without side reactions.

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EDIBLE FISH CONTAINING MARINE WAX ESTERS. A. A. Spark, Laboratory Analytical Services, Chromtek (Pty) Ltd., P.O. Box 4672, Cape Town, 8000, and P. Berman and E. H. Harley, University of Cape Town, Cape Town, South Africa.

Both *Ruvettus pretiosus* (the oil fish) and *Lepidocybium flavobrunneum* (the Escolar) are sold in South Africa under the name butterfish, and are considered delicacies, having a good texture and taste. However both fish contain large quantities (24%) of oil in their flesh, which, if consumed, is not digested. Investigations have established that the effect is not deleterious except perhaps on occasion to the dignity. Some thoughts on the role of wax esters in these fish, and attitudes to their consumption, will be presented.

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ANALYSIS OF WAX ESTERS IN MARINE ORGANISMS. J. R. Sargent and A. Fraser, Natural Environment Research Council, Institute of Marine Biochemistry, Aberdeen AB1 3RA, U.K.

The distribution and composition of wax esters in marine organisms, particularly calanoid copepods, euphausiids and some fish, will be outlined before reviewing methods currently used for the quantitative analyses of these lipids. The advantages and disadvantages of thin layer chromatography (TLC), flame ionization detection (Iatroscan) and TLC densitometry in quantitating lipid class composition will be discussed. The use of high resolution gas liquid chromatography in determining fatty alcohol and fatty acid compositions of marine wax esters will also be considered, emphasizing some problems of quantitation.

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MONOETHYLENIC ISOMERS OF FATTY ALCOHOLS AND ACIDS OF WHITE BARRACUDINA OIL. W. N. Ratnayake, A. Timmins and R. G. Ackman, Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, Box 1000, Halifax, N.S. B3J 2X4, Canada.

Information on the origin of the wax esters in marine lipids should be obtainable from a comparison of the respective monoethylenic isomers of fatty alcohols and fatty acids. Methodology suitable for the alcohols was adapted from that used for the monoethylenic fatty acids and will include a comparison of TLC technology based on mercuric adducts with that based on silver-nitrate plates. Oil from the white barracudina *Paralepis rissoi* kroyeri will be discussed as the type example.

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SPERM WAX AND DERIVATIVES ANALYSIS. Endo Fedeli and Mariani Carlo, Stazione Sperimentale Oli e Grassi, via Giuseppe Colombo, 79, 20133 Milano, Italy.

Sperm wax has been fractionated by TLC on Silica gel G; 2 main fractions have been obtained. The more polar of the 2 fractions was shown to be formed by triglycerides; the acidic fraction has been investigated by analysis of the methylesters obtained by transesterification. The less polar of the 2 fractions was shown to be formed by esters of long-chain acids and alcohols. Saponification separated the neutral and the acidic fractions and both have been characterized by GLC. Sperm wax sulphated derivatives have been desulphated and the esters fraction analyzed as shown before; the alcoholic fraction analysis, which does not undergo sulphation, can be used to characterize sperm wax sulphated derivatives. Fish oils used as sperm wax substitutes do not give a characteristic esters fraction.

Session P Flavor and Oxidation of Fats and Oils Tuesday a.m.

92

THE FLAVOR INTENSITY OF CARBONYL COMPOUNDS IMPORTANT IN FAT OXIDATION. Marvin Dixon and Earl G. Hammond, Iowa State University, Ames, IA 50011.

Carbonyl compounds were dissolved in mineral oil, the mineral oil emulsified into synthetic tap water and the emulsions stabilized with gum acacia. The flavor intensity of these emulsions were com-

pared with those of a series of standard dilutions of 2-heptanone, similarly emulsified. The intensities of individual 2-ketones, n-aldehydes, 2-enals, and 2,4-dienals were measured in terms of the 2-heptanone standard. Plots of the logarithm of a carbonyl's concentration vs the logarithm of the 2-heptanone concentrations giving the same flavor intensities gave straight lines. This allowed extrapolation of the lines to the threshold of 2-heptanone and the thresholds of the various carbonyl compounds. Such thresholds were comparable to those previously reported. The log-log plot for the various compounds versus 2-heptanone differed in slope, so the threshold is at best an approximate indicator of the flavor intensity of compounds at super threshold concentrations. The flavor intensity of mixtures similar to those found in oxidized soybean oil agreed fairly well with the added intensities of the components. The flavor intensities of such mixtures were similar to those of emulsions of oxidized soybean oil, but the quality of the flavor was not the same as oxidized oil.

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EVALUATION OF COOKING OIL PERFORMANCE OF HYDROGENATED SOYBEAN OILS BY SENSORY AND GAS CHROMATOGRAPHIC VOLATILE ANALYSES. E. N. Frankel, K. Warner, K. J. Moulton, Sr. and J. M. Snyder, Northern Regional Research Center, USDA, 1815 N. University Street, Peoria, IL 61604.

Soybean oil was continuously hydrogenated in a slurry system with copper (Cu) and nickel (Ni) catalyst to prepare different stocks of varying linolenate (Ln) content. All samples were bleached and deodorized and evaluated in the presence of 0.01% citric acid as metal inactivator. In room-odor evaluations carried out by heating oils to 190 C after frying bread cubes for various periods, our panel gave the oil hydrogenated with Cu to 2.4% Ln a significantly lower odor intensity score than the unhydrogenated control. The other oils hydrogenated with Cu to 0.5% Ln and with Ni to 4.6% and 2.7% Ln were not significantly better than the control. However, the oil hydrogenated with Ni to 0.4% Ln scored poorly because of its strong "hydrogenated-paraffin" odor. In evaluations carried out after heating and intermittent frying of bread cubes, our taste panel gave all samples fried in partially hydrogenated oil (2.2%, 2.7% and 4.6% Ln) a higher flavor quality score than the control sample fried in nonhydrogenated oil, which produced the fishiest responses. The bread cubes fried in highly hydrogenated oils (0.4% and 0.5% Ln) were not significantly better than the control, and they produced the most hydrogenated-paraffin responses. In contrast to the sensory evaluations, analyses by capillary gas chromatography (GC) showed that all hydrogenated oils after prolonged heating and bread frying produced less total volatiles than the unhydrogenated control oil. In conclusion, on the basis of results from our room-odor and fried-bread taste panels, all partially hydrogenated oils (2.4–4.6% Ln) performed better than the unhydrogenated control soybean oil. Although the most highly hydrogenated oils (0.4–0.5% Ln) showed the least volatile formation by GC, they were scored poorly by our panels because of the strong hydrogenated-paraffin odors they generated, especially the Ni hydrogenated oil.

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STRUCTURAL ELUCIDATION OF NOVEL ANTIOXIDANTS ISOLATED FROM ROSEMARY. Stephen S. Chang and Chi-Tang Ho, Rutgers, The State University, Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, New Brunswick, NJ 08903, and Christopher M. Houlihan, Lever Brothers Company.

A natural antioxidant was prepared by solvent extraction from dried, ground rosemary leaves. Following a vacuum steam distillation, this extract was fractionated using a 5 cm × 122 cm glass column packed with activated silicic acid. The column was eluted by a step by step gradient using 100% hexane as the initial eluent and then employing the following solutions of diethyl ether in hexane (E/H): 5% E/H, 10% E/H, 15% E/H, 25% E/H, 50% E/H and 75% E/H. The final eluent of this separation was 100% methanol. Two novel compounds, rosmariquinone and rosmaridiphenol, were further purified from 2 of the fractions. Structural elucidation of these compounds was accomplished by IR, MS, ^{1}H-NMR and ^{13}C-NMR spectroscopy. When tested in prime steam lard, the antioxidant activity of rosmariquinone and rosmaridiphenol were superior to BHA. (New Jersey Agricultural Experiment Station No.-K-10503-1-84.)

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THE EFFECT OF SELECTED PLANT STEROL ON HEATED SOYBEAN OIL. Lillian Armstrong and Pamela J. White, Iowa State University, 105 MacKay Hall, Food and Nutrition Department, Ames, IA 50011.

Soybean oil held at frying temperatures undergoes rapid deterioration caused by oxidation reactions. Certain plant sterols have been shown to slow these reactions, thus prolonging the life of the oil. In this study, soybean oil was heated with and without added sterol at 170 C, 180 C and 190 C for varying lengths of time. The sterol used was Δ^5 avenasterol and was isolated from oat oil. The amounts of individual fatty acids decreased dramatically during heating without Δ^5 avenasterol. These changes were slowed considerably with the presence of the sterol. Shifts in unsaturation of the fatty acids, which were monitored spectrophotometrically at 233 nm, decreased with the sterol addition. A high pressure liquid chromatographic method was devised to examine oxidative polymerization during heating. Large differences in the fingerprints of oils with and without added sterols were measured using wavelengths of 233 and 270 nm. Variations in the temperature of frying also resulted in some changes in the deterioration of the oil.

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DEGRADATION OF β-CAROTENE UNDER SIMULATED TIME AND TEMPERATURE CONDITIONS OF PALM OIL DEODORIZATION. Philip N. Onyewu and Henryk Daun, Rutgers—The State University of New Jersey, Department of Food Science, Cook College, P.O. Box 231, New Brunswick, NJ 08903.

A model system developed in our laboratory for the study of thermal degradation products (TDP) of carotenoids was employed. β-Carotene (10 g) in glycerol was heated at 210 C for 4 hr, 1 hr, 15 min and 5 min. The time and temperature conditions were chosen to include simulated deodorization of palm oil. In this study, the TDP of β-carotene were quantified as influenced by time and temperature of heating. Results indicate that at 210 C, degradation is almost complete after 4 hr and most of the nonvolatile products are viscous, yellow-brownish material. Shorter times (1 hr, 15 min and 5 min) cause less degradation. TDP include nonpolar as well as oxidized derivatives of β-carotene. The results of this study provide information on the type, amount and mechanism of formation of compounds generated from carotenoids during palm oil deodorization.

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THE EFFECTS OF ADDITIVES AND PACKAGING ENVIRONMENTS ON THE LIPID OXIDATION OF DRY MILK. David B. Min, Department of Food Science and Nutrition, The Ohio State University, 2121 Fyffe Road, Columbus, OH 43210, and Gary Reineccius, University of Minnesota, Minneapolis, MN.

Dry milk containing (a) no additive, (b) 100 ppm propylgallate and (c) 100 ppm propylgallate + 100 ppm sodium sulfite were prepared by centrifugal wheel atomization spray drying and then packaged in pouches containing air, 100% nitrogen or 92% nitrogen + 8% hydrogen and stored at 55 C and 65 C. The pouch was made of polyester, aluminum foil, ionomer, catalyst (palladium) and ionomer. The oxidation stability of dry milk was evaluated by measuring oxygen disappearance and volatile compounds formation in the headspace of a pouch using a gas chromatographic method. The volatile compounds were determined by the combination of gas chromatographic retention time comparisons with those of authentic compounds and mass spectrometry. The results were statistically analyzed by analysis of variance and linear regression equations were developed to predict flavor quality during storage. The results show that the dry milk stored under air gave the worst flavor quality and the product under 82% nitrogen + 8% hydrogen gave the best product. The result further suggested that the residual oxygen trapped by dry-milk particles seems to react with hydrogen

before oxygen reacts with fat in dry milk when palladium catalyst was impregnated between packaging materials.

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SHELF LIFE OF FRYING FATS FOR FAST FOOD SERVICES. Ying-Ying Gwo, Robert L. Ory, George J. Flick, Jr. and Harold P. Dupuy, Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

The fast-food industry has grown from 30 to 38 million dollars from 1981 to 1983 and faces a future of continued economic growth and market expansion. In order to provide food as economically as possible, the industry has been forced to reduce costs whereever possible. Initially, peanut oil was used as a frying fat but increased costs have caused more use of palm oil and animal-vegetable blended fats. Information relating to the stability and acceptability is limited and some shortenings are discarded too early, some too late. One chain reported that if their shortenings could be used 1 extra day, they could save over $500,000 per year. These studies were conducted on some chemical and physical properties that affect the quality and stability of frying fats under conditions similar to those in fast-food services: 10–11 hr/day at 360–370 F and exposure to air and light for 10-day periods. Two representative fats, a soybean oil and an animal-vegetable shortening, were cooked in open vessels at specified conditions, then sampled initially and after 2, 4, 6, 8 and 10 days. Samples were analyzed for development of peroxide value (PV), conjugated diene hydroperoxides (CDHP), gas chromatographic volatile profiles (GCV) and color development (CD), with and without ascorbyl palmitate (AP), an FDA-approved antioxidant, to try and retard autoxidation and deterioration of the fats. Chicken and french fried potato fats were obtained from a commercial outlet for comparison. Analytical results for PV, CDHP and GCV correlated well for all samples over the 10-day periods but GCV is the most useful method for measuring quality and deterioration of frying fats. AP, added once at the start and at 2-day intervals for 10 days, retards all deteriorative changes in both fats. It reduced PV, CDHP, GCV and CD a slight amount when added once but significantly reduced all undesirable changes, especially GCV and CDHP, when added every second day. Applications of these findings to extend the stability and quality of frying fats will be described.

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OXIDATION OF CHOLESTEROL IN HEATED TALLOW SYSTEMS. Thomas C. Ryan and J. Ian Gray, 300 Food Science, Michigan State University, East Lansing, MI 48824.

The oxidative stability of cholesterol (5-cholesten-3β-ol) in edible tallow employed as a deep-fat frying medium was investigated. Intermittent heating was found to be more detrimental than continuous heating to tallow cholesterol as well as to tallow triglycerides. Cholesterol present in tallow readily oxidizes under conditions used in frying operations. Thin layer chromatography (TLC) and gas liquid chromatography (GLC) analyses indicated that intermittent heating promoted more rapid changes in the oxidative state of cholesterol. Various oxides including 5-cholesten-3β,7α-diol, 5-cholesten-3β,7β-diol, 3,5-cholestadiene-7-one, and cholestan-3β,5α,6β-triol were identified in the intermittently heated tallow samples by 2-dimensional TLC. The effect of frying on the oxidative stability of intermittently heated tallow cholesterol via TLC indicated that cholesterol oxidizes in the system and that the oxidative derivatives of cholesterol are preferentially absorbed by french fried potatoes. 5-Cholesten-3β,7α-diol, 5-cholesten-3β,7β-diol, and 3,5-dholestadiene-7-one, as well as unoxidized cholesterol were identified in both tallow and french fried potato samples. Cholestan-3β,5α,6β-triol was also identified in trace amounts in french fried potato only. Commercially prepared french fried potatoes from a franchise using tallow as a frying medium were analyzed and found to contain 5-cholesten-3β,7α-diol, 5-cholesten-3β,7β-diol, 3,5-cholestadiene-7-one and unoxidized cholesterol.

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STABILITY OF RICE-BRAN OIL DURING DEEP FAT FRYING. Lucy Sun Hwang and I Mong Chiou, Graduate Institute of Food Science and Technology, National Taiwan University, P.O. Box 23-14, Taipei, Taiwan, R.O.C.

Rice-bran oil, lard, soybean oil, rice bran oil-soybean oil blend and rice bran oil-lard blend were heated separately at 190 ± 3 C for 8 hr each day for 6 days with and without the frying of instant noodles. Oil samples were taken periodically and analyzed for iodine value, dielectric constant, viscosity, acid value and the absorbance at 233 nm, in order to measure the deterioration of frying oils. Results of the analyses on iodine value, dielectric constant and viscosity showed that rice-bran oil was the most stable oil among these 5 oils during 48 hr of frying, followed by lard. Soybean oil was the least stable oil. The stability of rice bran oil-soybean oil blend and rice bran oil-lard blend was close to that of rice-bran oil. On the contrary, acid value showed the greatest increase for rice-bran oil and its blends, followed by lard, and showed the smallest change for soybean oil sample. During heating without frying foods, the stability of lard and rice bran oil-lard blend was the best of all 5 different oils, regardless of the analytical methods employed. The other oil samples showed similar tendency to those in the frying experiments.

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OXIDATIVE STABILITY OF SOY OIL AT DIFFERENT STAGES OF REFINING. Tai-Wan Kwon, Korea Advanced Institute of Science & Technology, P.O. Box 131, Cheong Ryang, Seoul, Korea, and Harry E. Snyder and Helen G. Brown, Food Science Department, University of Arkansas, Fayetteville, AR 72701.

A study of the changes in the oxidative stability of soy oil after various stages of refining was done by measuring the weight gain of small (1 g or less) samples incubated at 60 C. The most stable sample was crude soy oil. Based on time to reach a 1% weight gain, degumming decreased stability to 67% of the crude oil and alkali refining decreased stability to 33% of the crude oil. The refining steps of bleaching and deodorization had little effect (stability remained at 33% of crude), but partial hydrogenation increased stability to 67% of the crude oil. Preparation of a highly purified soybean triglyceride fraction decreased oxidative stability to only 10% of the crude oil. By adding varying amounts of tocopherols and phospholipids back to the purified soy oil, we learned that phospholipids alone had no effect on oxidative stability. In the presence of tocopherols, phospholipids were effective antioxidants, and stability could be brought back to ca. 80% of the crude oil. The method of weight increase led to the observation that the same oil gave different oxidation rates depending on the surface exposed. When the weight increase per unit weight was plotted against time, invariably the smaller weight samples oxidized faster than larger weight samples. This observation raises interesting questions about the importance of the surface and oxygen penetration into the oil and about the significance of oxidative measures such as peroxide values that are based on weight rather than exposed surface.

Session Q Surfactants and Detergents I: Performance and Evaluation Tuesday a.m.

102

EFFECTS OF ETHYLENE OXIDE DISTRIBUTION ON NONIONIC SURFACTANT PROPERTIES. K. W. Dillan, Union Carbide Corporation, Old Saw Mill River Road, Tarrytown, NY 10591.

Detergent-range primary alcohols are readily converted into effective nonionic surfactants by reaction with ethylene oxide. Under typical conditions, the products so obtained have broad ethylene oxide adduct distributions. Optimum performance properties are generally attained by varying the number of mol of ethylene oxide added to each mol of alcohol, by altering the length of the primary alcohol chain, or by varying the degree of branching. However, variations in the molecular weight distribution also affect performance properties, such that, products with relatively narrow ethoxylate-adduct distributions often possess features that are highly desirable in practical applications. For a given cloud point,

narrow-range ethoxylates contain less unreacted alcohol and fewer mol of ethylene oxide per mol of alcohol than conventional nonionic surfactants. Consequently, narrow-range ethoxylates have less odor, exhibit lower pour points, are more stable at elevated temperatures and exhibit lower aqueous viscosities and more limited gel regions than most conventional primary alcohol ethoxylates. Furthermore, narrow-range ethoxylates are generally more effective and efficient than broad-range ethoxylates, as evidenced by oil and water interfacial tension values, adhesion tensions, wetting data, and oily soil detergency results. Other areas where the effects of the ethylene oxide distribution are evident include foam stability, solvent solubility, and emulsification phenomena.

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DETERGENCY PERFORMANCE COMPARISONS BETWEEN LAS AND ABS USING CALCIUM SULFONATE PRECIPITATION BOUNDARY DIAGRAMS. K. Lee Matheson, Conoco Inc., P.O. Box 1267, Ponca City, OK 75603.

Detergency performance studies show that, at high-use concentrations, LAS tends to be less sensitive to hardness than ABS. An explanation for this behavior can be obtained from the precipitation boundaries for LAS and ABS on their calcium sulfonate phase diagrams. Such precipitation boundary diagrams appear to be useful in predicting practical detergency performance behavior at a wide range of hardness levels and use concentrations.

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INTERACTIONS BETWEEN LAS AND NONIONICS. Ted P. Matson, Nelson F. Borys and Michael F. Cox, Conoco Inc., P.O. Box 1267, Ponca City, OK 74603.

Physical interactions between linear alkylbenzene sulfonate (LAS) and various linear alcohol nonionics (NI) have been determined. The effect nonionic has on LAS with respect to critical micelle concentration, surface and interfacial tension and water hardness sensitivity is dependent on both hydrophobe and hydrophile structure. A large negative β parameter (strong molecular interaction) exists for these systems based on large reductions in critical micelle concentrations and surface and interfacial tensions. We have also determined that the interaction between hardness ions and LAS can be greatly altered by correctly choosing the hydrophile and hydrophobe of the NI surfactant. LAS and NI mixtures were also investigated in typical heavy-duty powdered formulations. The data indicate that correlating detergency performance to LAS/NI physical properties is possible.

105

THE DEVELOPMENT OF AN OPTIMIZED HEAVY-DUTY LIQUID CONTAINING FABRIC SOFTENER. Connie Lange Merrill, Shell Development Company, P.O. Box 1380, Houston, TX 77001.

The heavy-duty liquid containing fabric softener (HDL/FS) provides, in one package, detergency, fabric softening, and antistatic control. However, the redeposition of soils onto fabric surfaces during the laundering process is a recognized problem when using an HDL/FS. As the fabric softener (cationic surfactant) molecules adhere to the fabric surface, imparting softening and antistatic properties, soils can become trapped on this coated surface and the fabric will appear dingy and yellow. Thus, the key to a successful HDL/FS formulation is the effective control of soil redeposition, in addition to maintaining a high level of soil removal. The effect of anionic surfactant in HDL/FS on detergency and soil redeposition over multiple wash cycles was monitored by varying the percentage of weight ratio of nonionic (alcohol ethoxylate)/anionic in these formulations. The addition of the anionic to the all-nonionic HDL/FS significantly lowered sebum and clay soil redeposition. However, these nonionic-anionic HDL/FS exhibited ca. 10% decrease in overall detergency relative to the all-nonionic HDL/FS. Introduction of a topped alcohol ethoxylate (AEO) into an HDL/FS provided a higher level of detergency compared to a conventional AEO. Thus, the combination of a topped alcohol ethoxylate with an anionic surfactant in an HDL/FS results in an optimized formulation that softens, eliminates static cling, maximizes cleaning, and minimizes soil redeposition on fabric.

106

PEAKED DISTRIBUTION ETHOXYLATES—THEIR PREPARATION, CHARACTERIZATION, AND PERFORMANCE EVALUATION. K. Lee Matheson and Kang Yang, Conoco Inc., P.O. Box 1267, Ponca City, OK 74603.

A peaked ethylene oxide distribution can be produced in alcohol ethoxylate using new catalyst systems. The mechanism of ethoxylation and the theoretical prediction of ethylene oxide distribution will be discussed in detail. Such ethoxylates are markedly different from a conventional ethoxylate in physical properties and performance characteristics. Performance and formulation studies show several advantages for the peaked distribution in typical household product formulations.

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REDUCTION IN PUMPING DURING THE SPRAY DRYING OF A NONIONIC-BASED, HEAVY-DUTY POWDER. D. L. Wharry*, E. L. Sones, S. E. McGuire, Conoco Inc., P.O. Box 1267, Ponca City, OK 74603, and J. Lovas, Lever Brothers Company.

The plume observed during the spray drying of a nonionic-based, heavy-duty powder has been attributed to the volatilization and recondensation of unethoxylated alcohols and other components in the alcohol ethoxylate. These volatile components comprise only 24–40% of the particulate emissions, but they are responsible for 85–95% of the observed opacity. Therefore, the relationship between the observed opacity of the plume and particulate loading is not valid for the exhaust air from a spray tower producing nonionic-based powdered detergents. Alcohol ethoxylates are not decomposed to any measurable amount in a typical spray-dryer operation. The pluming tendency of alcohol ethoxylates depends exclusively on their relative vapor pressures. The vapor pressure of an alcohol ethoxylate is a function of the hydrophobe composition (starting alcohol) and the degree of ethoxylation, which determines the level of unethoxylated alcohols. A new generation of alcohol ethoxylates with 50% less unethoxylated alcohol for a given ethoxylation level has recently been introduced. These Novel™ alcohol ethoxylates result in drastically improved pluming characteristics.

Session R Oils, Proteins and By Products from New Crops Tuesday a.m.

108

GENERIC PROBLEMS OF DEVELOPING AND MARKETING NEW OILSEED CROPS. Eugene B. Shultz, Jr., School of Engineering and Applied Science, Box 1106, Washington University, St. Louis, MO 63130.

The development of a new crop is probably much more challenging than most observers realize. Funds are not readily available, and convincing established growers and processors to support new crop development is difficult because of the substantial uncertainties usually perceived. The problem is further complicated if the market as well as the new crop requires development. Further, the industrial infrastructure may be incomplete and will need to be provided. No complete source seems to be authorized as well as prepared to carry out all major subtasks. For example, the U.S. Department of Agriculture does new crop-related research, but is not allowed to carry out use research on novel seed oils to stimulate industrial uses and secure markets for the new oils. In the area of new cropping practices needed for many novel oilseed crops suited for marginal lands, it is not clear that agronomists will quickly turn away from conventional cropping problems on prime farmland to the difficult tasks of marginal land farming. Government or other funding may or may not be readily available to support the necessary agronomic and ecological research and development where issues of marginal land farming are involved, such as preserving the long-term stability of the land. Illustrations will be drawn from experiences with dry- and wetland novel oilseed cropping studies presently underway in the US.

Meetings

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CRITICAL AGRICULTURAL MATERIALS. Richard Wheaton, USDA, Domestic Rubber Program, Room 446A Administration Building, 14th and Independence N.W., Washington, D.C. 20250.

Dependence on foreign sources for certain strategic and essential industrial materials is a growing national concern. Forecasts beyond the end of this century indicate that drastic reductions of petroleum supplies will affect the nation's ability to produce numerous hydrocarbon-based products for industry and defense. Traditional sources of hydrocarbon products are nonrenewable, and will rapidly increase in cost over the next 3 decades. Alternative sources of hydrocarbons provide opportunities for agriculture. Research has shown that agricultural plants can provide hydrocarbon sources for manufacture of products currently produced from petrochemicals. The Native Latex Act of 1978 (P.L. 95-592), established a national policy for addressing the production of hydrocarbons from agricultural plants. In 1983, Congress acted on legislation with the title of *The Critical Agricultural Materials Act*. This interest of our national government in agriculture-produced hydrocarbons and other plant products has the support of the departments of Agriculture and Defense, and the National Science Foundation, Federal Emergency Management Agency and General Services Administration. As this interest grows, the Department of Agriculture will increase assistance in the development of agriculturally produced hydrocarbons. The present success in this area is demonstrated in the production of natural rubber from guayule. Successful agronomic production and chemical extraction of rubber, as well as by-products, indicates the contribution that agriculture can make to materials critical for industrial uses and national defense.

110

NEW CROPS FOR CHEMICALS, FUELS AND MATERIALS. Marvin O. Bagby, USDA-ARS-NRRC-NAEC, 1815 N. University, Peoria, IL 61604.

During the past century, US agriculture has developed a handful of new crops, perhaps best exemplified by the soybean. Guayule, grown for rubber during a short period and then abandoned, now is being reevaluated as a domestic source of rubber. In 1956, the USDA established a program to identify plant species with potential as annually renewable sources of fiber for pulp and paper and as industrial oilseed crops. Many promising species were identified that satisfy the major objectives, and several species are undergoing developmental research. One of these, kenaf, is now a crop for papermaking in Thailand. In 1974, the USDA initiated a search to identify plant species that accumulate large amounts of easily extractable, high-energy compounds suitable for chemicals, fuels and petroleum-sparing feedstock for manufacture of materials. Plant families from which more than one promising species has been identified thus far are Anacardiaceae, Asclepiadaceae, Campanulaceae, Caprifoliaceae, Compositae, Elaegnaceae, Euphorbiaceae, Garryaceae, Labiatae, Leguminosae and Solanaceae.

111

DEVELOPMENT OF NEW HYDROCARBON SOURCES. Robert P. Adams, Bio-Renewables Institute, Inc., 2502 Milfoil Cove, Austin, TX 78704.

Eight new sources of hydrocarbons are reviewed. The sources, composition (products), processing needs and potential economic opportunity for oilseed processors are discussed. The sources examined are guayule, jojoba, meadowfoam, neem, gumweed, milkweed, candelilla and *Dunaliella*. The products from these sources are rubber and resin (guayule), long-chain wax esters (jojoba), long-chain triglycerides for synthetic lubricants (meadowfoam), insecticides (neem), diterpene acids for resins (gumweed), triterpenoids for chemical feedstocks (milkweed), waxes (candelilla) and glycerol and β-carotene (*Dunaliella*). Processing needs range from traditional solvent (jojoba, meadowfoam) and sequential multiple solvent extraction (neem), to whole plant solvent (gumweed, candelilla), ground, whole plant multisolvent extraction (guayule, milkweed) and single-cell lysing/extraction (*Dunaliella*). The economic potential for oilseed processors is evaluated for each of these crops. A close correlation is suggested between the degree of product specialization and potential processing markets.

112

NEEM SEED AS A NATURAL SOURCE OF PESTICIDAL COMPOUNDS. Martin Jacobson, USDA, Biologically Active Natural Products Lab., Beltsville Agricultural Research Center, Beltsville, MD 20705.

Crop loss from feeding by insect larvae and adults is estimated to total billions of dollars each year in the US and is even higher in developing countries. Safe, biodegradable substitutes for chemical insecticides must be found. All parts of the neem tree, *Azadirachta indica* A. Juss (family Meliaceae), which is native to the tropics in cultivated and wild plantations of India, Pakistan, East and West Africa and the Caribbean countries, possess insecticidal and repellent properties; trees are virtually free of insect pests. The seeds contain 42–50% of oil that is burned for light and heat and has potential for use in production of waxes, lubricants and aromatic chemicals. Azadirachtin, a complex sesquiterpenoid that is the major insecticidal component of the seed, was shown to repel and prevent or drastically reduce feeding by at least 40 species of pest insects and nematodes, was effective at concentrations as low as 0.1 ppm, and appeared to be safe to beneficial insects, fish, animals and most crop plants. Neem is a fast growing, sturdy tree that can be established without irrigation in hot and dry regions of the world where it grows well on poor, shallow, stony or sandy soils. The USDA and the State Department's Agency for International Development have initiated programs to grow neem as a crop in southern Florida, the Caribbean islands and Central and South America.

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POLYMER NETWORKS INVOLVING BOTANICAL OILS WITH SPECIAL FUNCTIONAL GROUPS. John A. Manson, Lehigh University, Department of Chemistry, Materials Research Center, Bethlehem, PA 18015.

For some time we have been studying the modification of plastics with botanical oils containing functional groups. For example, castor and vernonia oils contain hydroxyl and epoxy groups. Other oils, such as linseed and crambe, can be readily epoxidized. Some of these oils are of particular interest because they are derived from plants that can be grown in arid and semiarid areas. Alesquerella species, commonly known as bladderpod or popweed, grows well in the southwestern US. Crambe is also of interest as a new source of industrial oil. By crosslinking the oils and combining with polystyrene to form interpenetrating polymer networks, preparing a series of polymers ranging from tough plastics to leathery materials to reinforced elastomers was possible. In addition, some of the epoxidized oils can be used to toughen some epoxies without significant loss of stiffness. This paper discusses what is known about the new cultivars with oils that have special functional groups, reviews technical progress to further modify them and suggests future possibilities for other unique modifications of oils and their potential commercial uses.

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THE CHINESE TALLOW TREE AS A SPECIALTY OILSEED CROP. H. W. Scheld, Department of Biology, University of Houston, Houston, TX 77004.

The Chinese tallow tree is being studied as a potential new crop for the southeastern US, a source of 2 distinctly different and separately recoverable seed fats. The hard seed shell has a arillike covering consisting of crystals of a hard white tallow embedded in a fibrous matrix. The seed kernel inside of the impermeable shell produces an oil. The unique properties of these 2 products suggest that the Chinese tallow tree could become a useful source of high-value, specialty vegetable oils. The tallow consists predominantly of the triglyceride palmitic-oleic-palmitic (POP), and up to 98% of the tallow fatty acids are palmitic and oleic acids. The oil is highly unsaturated (iodine number 170-180), containing up to 47% linolenic acid and 10% or more short-chain acids (8-hydroxy-5,6-octadienoic acid and 2,4-decadienoic acid) linked in a tetraester "estolide" triglyceride. Comparative analysis of tallow and oil from a large number of individual trees indicates remarkable quantitative and qualitative differences in tallow and oil content and the relative

proportions of the different chemical species. Such variability suggests the possibility for selection and propagation of particular tree strains to supply specific market requirements.

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AGRONOMIC POTENTIAL AND SEED COMPOSITION OF *Cuphea*, A NEW OIL CROP. Frank Hirsinger, Department of Crop Science, Oregon State University, Corvallis, OR 97331.

Different species of the genus *Cuphea* (Lythraceae, loosestrife family) produce high percentages of medium-chain fatty acids in their seeds. Four different fatty acid patterns (up to 68% caprylic acid, 87% capric acid, 78% lauric acid, or 63% myristic acid) are present in that genus at oil percentages of 16–42%. The seed protein content varies between 15 and 30%. Until now, feeding experiments with rats have not indicated any toxic byproducts in *Cuphea* meal. Some *Cuphea* species could very well substitute for coconut or palm kernel oil, whereas others offer an interesting source of highly concentrated capric fatty acid. For acclimatization and domestication of wild *Cuphea* species, agronomic research must continue with different *Cuphea* species. This research has recently been initiated in the US. Field experiments have shown that the yield potential is ca. 300 kg oil/ha.

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KOCHIA SEEDS AS A SOURCE OF PROTEIN AND OIL. Krystyna Sosulski and Ewen C. Coxworth, Saskatchewan Research Council, 30 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 0X1, and Frank W. Sosulski, University of Saskatchewan.

Kochia (*Kochia scoparia*) is an uncultivated annual species that has been widely investigated as a potential crop for marginal and saline soils. Although the seeds of kochia are small, the yielding potential of the plant exceeds that of wheat. The oil and protein contents of the intact seeds are 13–15% and 25–30% of the dry matter. The seeds contain bracts, which, when removed, would enhance the oil content to a level that could be extracted commercially. The triglycerides are composed primarily of 10% palmitic, 20% oleic and 60% linoleic acids, indicating a useful balance of shorter-chain saturated and polyunsaturated fatty acids. The meal proteins contain 5% of lysine and 3% of sulfur containing amino acids and are well balanced in other essential amino acids.

Session S Poster presentations Tuesday a.m.

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EFFECT OF THE THERMAL PROCESS ON SOME NUTRITIONAL AND FUNCTIONAL CHARACTERISTICS OF PROTEIN ISOLATES FROM SESAME SEED PASTES. F. M. Báez, and A. L. Arjona, Departamento Ciencia y Tecnología de Alimen tos del Instituto Nacional de la Nutrición "Salvador Zubirán", Vasco de Quiroga N°15, Col. Tlalpan, C.P. 14000, México, D. F., and C. J. Camacho, Centro de Graduados e Investigación del Instituto Tecnológico de Veracruz, Veracruz, México.

Different oil extraction processes for sesame seed produce residual pastes with low nutritional values because of thermal damage but still with high levels of antinutritional factors such as phytates, oxalates and crude fiber. A protein isolation technique was applied for reducing the antinutritional factors existing—rendering a food grade product. Three different pastes were used: I-1 prepressed hexane extracted at 90 C; I-2 industrial expeller paste treated at 110 C; I-3 hexane extracted in mild conditions (40 C) and used as control for the experiment. The isolation parameters were: 40 C, 1 M NaCl ionic strength, 30 min stirring, maximum protein solubilization pH values 11–13 and pI 5. Resultant isolates presented a protein concentration of 93%, 89% and 90% for I-1, I-2 and I-3; antinutritional factors were eliminated in all the samples at a rate greater than 82%. Thermal treatments for I-1 and I-2 decreased the protein solubility, oil-emulsifying and water-absorption capacities in a 66%, 18% and 61% compound with I-3. Net protein utilization (NPU) values were 28%, 17% and 39% from the casein NPU, for I-1, I-2 and I-3, respectively. The isolates presented a lysine chemical score (compared with FAO '73 lysine values) of 26%, 43% and 44% for I-1, I-2 and I-3. Large amounts of sulfur amino acids and tryptophane were found—170–220% and 66–138% for the resultant isolates.

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MODE OF ENTRY OF *Aspergillus flavus* INTO COTTON TO CAUSE AFLATOXIN IN SEED: EXTERNAL OR INTERNAL? L. S. Lee and M. A. Klich, USDA, ARS, Southern Regional Research Center, PO Box 19687, New Orleans, LA 70179.

Dogma relating to *Aspergillus flavus* invasion of cotton and formation of aflatoxin in seed contends that infection is external, following insect damage of developing bolls. However, seed infected with *A. flavus* have been detected in bolls with no apparent damage of any sort. Inoculations of cotton plants at several natural openings resulted in *A. flavus* seed infection rates significantly higher than in controls. Lint from some of these seed exhibited the bright green-yellow (BGY) fluorescence associated with *A. flavus*. *A. flavus* has been detected in peduncles supporting naturally contaminated bolls, indicating systemic infection. Conversely, all seed from locks in unopened bolls inoculated through a puncture-wound in the carpel wall had BGY fluorescent linters. On opening, the appearance of locks in these wound-inoculated bolls closely resembled that of naturally contaminated bolls. The percentage of seed contaminated with aflatoxin following external inoculation of bolls was comparable to that found in naturally contaminated bolls. BGY fluorescence was detected on external lint in locks rather than throughout the lock or just around the seed. Environmental conditions in high toxin-producing areas probably permit both systemic (internal) and nonsystemic (external) invasion by *A. flavus* of cotton.

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AMMONIATION OF AFLATOXIN B_1 IN A PRESSURE CHAMBER USED TO DECONTAMINATE TOXIN-CONTAINING COTTONSEED MEAL. L. S. Lee, S. P. Koltun and J. B. Stanley, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Radio-labeled aflatoxin B_1, mixed with nonlabeled aflatoxin B_1 distributed on an inert carrier, was treated along with cottonseed meal in a pressurized ammoniation chamber with 4% ammonia at 40 psi, at 100 C for 30 min. Twenty percent of the radiolabel was lost following ammoniation. Less than 1% of the toxin remained unchanged; the rest was converted to degradation products. Approximately 30% was converted to compound with m. wt. 206, a nonfluorescent phenol that retains the difuran moiety but lacks both the lactone carbonyl and the cyclopentenone ring characteristic of aflatoxin B_1. The remaining 70% reaction product contained only fragments of aflatoxin B_1 having molecular ions less than 200.

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INFLUENCE OF FUNGAL INFECTION ON WHEAT KERNELS, SOYBEAN SEEDS AND SESAME SEEDS. R. S. Farag, F. A. Khalil and A. E. Basyony, Faculty of Agriculture, Cairo University, Giza, Egypt.

The presence of various fungi in wheat kernels, soybean seeds and sesame seeds obtained from different localities in Egypt was studied. The most widely distributed fungi were *A. flavus* and *F. solani*. The least infected crop during storage was sesame, followed by soybean. The fungi caused significant changes in the chemical composition of the kernels and seeds under study. Values for refractive index, acid value, saponification value, iodine value and unsaponifiable content of oils extracted from kernels and seeds that were deliberately infected by the most widely distributed fungi were greatly influenced, particularly the acid value.

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DETECTION OF HEXANE AND OTHER VOLATILE HYDROCARBONS WITH COATED PIEZOELECTRIC CRYSTALS. T. J. Jacks and T. P. Hensarling, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

We have constructed a piezoelectric crystal detection system for measuring certain volatile hydrocarbons such as hexane. Our piezoelectric crystals are quartz that electronically vibrate at 9 megahertz. We monitor this vibration. The crystals respond to a change in mass, i.e., a decrease in frequency of 400 Hz per μg of increased mass. The crystals are coated with selective adsorbants so that only volatiles of choice are readily adsorbed for an increase in mass. When these volatiles are passed over the coated crystal, the frequency decreases as the volatile is adsorbed, reaches a minimum, then returns to the original frequency as the volatile desorbs. Little response occurs with other volatiles. The coating, in this case squalane for hexane and related volatiles, provides the selectivity. Theoretically, 10^{-12} grams can be measured. The workings of this system will be described and discussed.

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INFLUENCE OF THE SOURCE OF DIETARY FAT ON BLOOD SERUM LDL/HDL RATIOS IN THE RAT: A CROSSOVER STUDY. R. E. Worthington and Josephine Miller, Department of Food Science, University of Georgia Experiment Station, Experiment, GA 30212.

Post-weanling female rats were maintained on a standard 5% fat diet until they weighed ca. 70 g and were then allotted to 1 of 4 test diets on the basis of body weight. The test diets contained 1 of 2 levels (10% or 25%) of either peanut oil (PO) or corn oil (CO). At the end of the first experimental period (41 days), the diet of each group of rats was switched from 1 oil to the other at the same dietary level for the second period of 37 days. Finally, all rats were returned to the standard diet for 35 days. Blood samples were taken at the end of each experimental period for serum lipoprotein electrophoresis and to determine the ratio of low-density lipoproteins to high-density lipoproteins (LDL/HDL). The analysis of variance indicated that LDL/HDL was significantly higher ($P \leq 0.001$) for PO (3.9) than for CO (2.4). The LDL/HDL ratio tended to increase with age of the rats, and was significantly ($P \leq 0.05$) higher at 3.7 in period 2 than at 2.6 in period 1. However, the interaction between type of oil and period was not significant, indicating that the effect of type of oil was not caused by the age of the rats. Difference from concentration of oil in the diet was also significant ($P \leq 0.05$) but interactions with type of oil and experimental period were not significant. After the final period, LDL/HDL was not significantly different among the treatment groups (average 0.86).

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POSSIBILITIES AND LIMITATIONS OF THE GAS CHROMATOGRAPHIC SEPARATION OF TRIGLYCERIDES WITH CAPILLARY COLUMNS. E. Schulte, Institut fur Lebensmittelchemie, University of Muenster, Piusallee 7, D-4400 Muenster, West Germany.

The separation of triglycerides by gas chromatography (GC) using short packed columns according to the C-number was described for the first time about 15 years ago and in many publications thereafter. However, this method did not find broad practical application. In the last 5 years, separations by capillary GC have been reported. The advantage of this method consists in a better resolution and shorter retention times with short capillaries. With longer columns (10 m and more), fats can be separated not only by the chain lengths of the fatty acids in the triglycerides, but also by the number of unsaturated fatty acids in the molecules. However, higher unsaturated fats give a bad resolution and calibration for a quantitative evaluation is not easy. This method has, therefore, found only limited practical application. The usefulness of the method for some fats is shown. The higher unsaturated fats can be analyzed quantitatively according to chain length only after complete hydrogenation with a Pt catalyst. Fats with short fatty acids show peak splitting caused by different chain-length distributions. This effect is investigated. A simple calibraton procedure with the aid of a homologous series of triglycerides has been developed.

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ENZYMIC SYNTHESIS OF CARBOHYDRATE ESTERS OF FATTY ACID. Hajime Seino and Tsuyoshi Uchibori, School of Hygienic Sciences, Kitasato University, Kitasato 1-15-1, Sagamiharashi, Kanagawa 228, Japan, and Toshiyuki Nishitani and Sachiko Inamasu, Dai-Ichi Kogyo Seiyaku Co., Ltd., Japan.

Attempts were made by the authors to synthesize carbohydrate esters of fatty acids enzymatically with view to overcoming the problems associated with the chemical process for the synthesis of commercial sucrose esters. The enzymes used were the microbial lipase from microorganisms belonging to Rhizopus, Enterbacterium, Aspergillus, Pseudomonas, Chromobacterium, Candida, Mucor, and Penicillium. Fatty acids (stearic, oleic and linoleic acid) and carbohydrates (glucose, fructose, sucrose and others), which were used for the enzyme reactions, were obtained from commercial sources. The enzyme reaction was carried out by the following method. The enzyme and substrates were mixed in a flask and incubated at 37 C for 72 hr. After freeze-drying and extracting the mixture, the reaction products were subjected to thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC). We observed by TLC and HPLC that carbohydrate esters were produced by the enzyme reaction. And these observations were confirmed by IR, NMR and mass spectrometry. The lipase from the microorganism belonging to Candida had the most enzyme activity on the synthesis of carbohydrate esters. The optimum pH for the activity of the enzyme on the synthesis of carbohydrate esters was found to be in a range from 5.0 to 7.0 in phosphate buffer and maximum activity was observed at pH 5.4.

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LIPID, STEROL, CHOLESTEROL AND FATTY ACID CONTENTS OF SQUID, OYSTERS, CLAMS AND MUSSELS. Robert G. Jensen and Richard A. Ferraina, University of Connecticut, Department of Nutritional Sciences, Storrs, CT 06268.

Squid, oysters, clams and mussels obtained from Long Island Sound were analyzed for total lipids, sterols and cholesterol contents. The lipids were obtained by Folch extraction and the amounts were determined gravimetrically. The sterols were recovered after saponification of the lipids, silylated and determined by gas-liquid chromatography (GLC), using an internal standard. Cholesterol and several other sterols were identified. The total fatty acids were analyzed by GLC. The amounts found were: percentage total lipids, total sterols and cholesterol (mg/100 g wet weight): squid (n=4), 2.5 and 160; oysters (n=2) 3.0, 66.5 and 33.6; clams (n=3) 2.1, 84.9 and 32.0; mussels (n=4) 2.0, 66.5 and 23.9. The fatty acids contained relatively large quantities of polyunsaturates, particularly 20:5.

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) OF THE CAROTENOIDS IN CORN GRAIN. Evelyn J. Weber, USDA-ARS-NCR, S-320 Turner Hall, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

Two general classes of carotenoid pigments, carotenes and xanthophylls, are primarily responsible for the yellow color of corn grain. The carotenoids are important feed constituents of corn because carotenes are precursors of vitamin A and xanthophylls impart a desirable yellow color to egg yolks and the skin of poultry. The carotenoids also function as antioxidants. Although the inheritance of carotenes and xanthophylls in corn has been extensively studied and many mutants have been observed, the genetics and pathways of biochemical synthesis are not fully understood. The greatest problem has been the complex and time-consuming analysis required to quantitate the individual carotenoids. Five carotenes and 4 xanthophylls have been identified as the major carotenoids in corn grain, along with many minor components. We have developed an HPLC method to separate the carotenoids. After extraction from the corn grain and saponification, the sample was run on a normal phase column with hexane-isopropanol as the mobile phase. The carotenoids were identified by the selection of appropriate detection wavelengths from 276 nm to 480 nm. Chromatograms of the carotenoid separations will be shown along with the corn phenotypes.

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THE OIL COMPOSITION OF TWO ANNUAL SPURGES. P.

Caldeira Polyzou, J. B. M. Rattray, J. F. Alex and G. W. Anderson, Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

A general oil content (petroleum spirit-soluble material) of 3–8% of the biomass has been determined in *Euphorbia helioscopia* and *E. peplus*. Variations existed with plant growth stage and cultivation practices. The oil of both plants had an energy value comparable to that of gasoline. Nonsaponifiable matter (NM) was found to account for 60–85% of the recovered oils. The occurrence of the fatty acid component varied inversely with NM and was characterized by a content of 18:3 acid amounting to greater than 50% of the total acid. The oil of mature *E. helioscopia* had a percentage composition of several identified long-chain hydrocarbons (<1), wax and carotenoid material (15), triterpenoid esters (31), a complex fraction (40) containing unidentified triterpenoids, free fatty acids and free aliphatic alochols and polar material (13) containing sterols, xanthophylls and pigments. Specific examination of NM of *E. peplus* revealed a percentage composition of hydrocarbons (11), various saturated and unsaturated alcohols (64) including ceryl alcohol, sterols and tetra- and pentacyclic triterpenoids, and highly unsaturated/polar material (25). Based on various chemical analyses, the possible industrial value of these oils has been considered.

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PHYSICAL, CHEMICAL AND FUNCTIONAL PROPERTY CHANGES IN STORED SOY PROTEIN CONCENTRATE AND SOY-PROTEIN ISOLATE. Mimg Jong Kaing and Mark H. Love, Food and Nutrition, Iowa State University, 110 Mac Kay Hall, Ames, IA 50011.

Commercially processed soy-protein products were humidified over saturated salt solutions (0%, 11%, 21%, 32% and 48% RH) at 40 C to produce samples of varying water activities. The influence of water activity on the physical, chemical and functional properties of soy-protein products during 3 months of storage was investigated. The results showed that no significantly ($p>.05$) different changes in crude-protein content, digestibility, total lipids, U/S ratio, water-absorption capacity, monolayer moisture, foam capacity and color occurred. However, sulfhydryl contents ($p<.05$) and nitrogen solubilities ($p<.01$) decreased significantly at the water activities of 32% RH and higher after 3 months of storage. Strong correlations were found between the change in sulfhydryl content and nitrogen solubility (0.46–0.97), sulfhydryl content and foam capacity (0.79–0.99), and nitrogen solubility and foam capacity (0.23–0.97). Strong correlations were also found between the pH of the test and nitrogen solubility (0.90–0.99), the pH of the test and foam capacity (0.74–0.93), and nitrogen solubility and foam capacity (0.60–0.91) in the stored products. The results suggested that the effect of water activity on nitrogen solubility and sulfhydryl content were most detrimental, which in turn affect the functional properties of these soy-protein products.

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THE SOLUTION TO SPENT CATALYST DISPOSAL–RECLAMATION. Frank J. Hennion, Inmetco, P.O. Box 720, Ellwood City, PA 16117, and Vincent A. Vellella, Pittsburgh Pacific Processing Co.

Since 1982, the Pittsburgh Pacific Processing Company (PPPC), a subsidiary of Inmetco, has been processing and reclaiming nickel from spent hydrogenation catalyst. Our presentation will schematically depict the processing of spent nickel catalyst at the Pittsburgh Pacific Processing facility. Although an energy efficient and environmentally sound process is at the heart of the processing circuit, feed material and nickel accountability have been essential for the success of this business venture. Processing highlights will illustrate the many facets of accountability (drum storage, inventory control, sampling and assays), processing flow diagrams and the use of the nickel concentrate end product.

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THE EFFECT OF DIETARY *trans* FATTY ACIDS ON ENERGETIC EFFICIENCY AND MITOCHONDRIAL FUNCTION. Remi de Schrijver, University of Ghent, 19, Heidestr aat, 9220 Merelbeke, Belgium, and Orville S. Privett, The Hormel Institute, University of Minnesota, 801 – 16th Avenue N.E., Austin, MN 55912.

Feeding experiments with rats were conducted to study the effect of dietary 18:1-t and 18:2-tt on oxidative phosphorylation of isolated liver mitochondria and on energetic efficiency as measured in energy balance experiments. Partially hydrogenated soybean oil (PHSO) with 45% 18:1-t and an ethyl ester concentrate of *trans* fatty acids (TRANS) containing 52% 18:2-tt were used. Six groups of male rats were fed diets with 10% (w/w) safflower oil (SAF), 8% PHSO + 2% SAF, 0.5% TRANS + 9.5% SAF, 1% TRANS + 9% SAF, 2% TRANS + 8% SAF and 5% TRANS + 5% SAF, respectively. Dietary fat composition did not affect metabolizable energy values. Compared with the SAF group, rats receiving PHSO showed no different effect on energy use or mitochondrial respiratory function. TRANS reduced in vivo energetic efficiency at the 5% level, while mitochondrial ATP synthesis was significantly depressed in both the 2% and the 5% TRANS groups. A parallel was found between 18:2-tt incorporation in liver mitochondria, reduced mitochondrial oxidative function and depressed energetic efficiency. The higher heat expenditure in the 5% TRANS group was associated with less efficient use of metabolizable energy for maintenance, while the basal metabolic rate was unaltered. The experiments indicated that 18:2-tt exerted a significantly negative effect on energy use at the 2% and 5% TRANS levels, even when fed with relatively high concentrations of linoleate.

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INFLUENCE OF SUPERCRITICAL CARBON DIOXIDE ON PROTEINS. Juergen K. P. Weder, Institut fuer Lebensmittelschemie, Technische Universitaet Muenchen, Lichtenbergstr. 4, D-8046 Garching, Federal Republic of Germany.

The use of supercritical carbon dioxide for food extraction is expanding. In order to determine the influence of supercritical carbon dioxide on food constituents under extraction conditions, the effects on proteins and amino acids have been studied. The results, with lysozyme as a model protein, are presented here. Commercial lysozyme was treated in an autoclave with humid supercritical carbon dioxide and nitrogen (300 bar, room temperature for 6 hr and 80 C for 2 hr and 6 hr). Amino acid analysis and assays of TNBS-reactive lysine failed to show any alteration in any of the samples. Independent of the gas used, the samples treated at 80 C were digested by trypsin better than the untreated sample or the samples treated at room temperature. The digestibility increased with exposure time because of an unfolding of the protein molecules. Reductive and nonreductive SDS-polyacrylamide gel electrophoresis indicated partial oligomerization of the protein molecules mainly caused by disulfide interchange as well as some fragmentation because of hydrolysis of peptide bonds. From studies reported elsewhere, heating of proteins in the presence of water has been shown to be responsible for the same type of alterations observed here with supercritical carbon dioxide treatment. The unfolding of protein molecules as well as oligomerization and fragmentation to the extent observed here do not negatively influence protein use. Chemical reactions of carbon dioxide with proteins, as well as deteriorations, could not be demonstrated under the reaction conditions used in supercritical carbon dioxide extraction of foods.

Session T The Regulation of Production of Prostaglandins and Other Eicosanoids Tuesday p.m.

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ROLE OF ARACHIDONOYL-CoA SYNTHETASE IN EICOSANOID PRECURSOR UPTAKE AND RELEASE. Ellis J. Neufeld and Philip W. Majerus, Division of Hematology-Oncology, Washington University School of Medicine, 600 South Euclid, St. Louis, MO 63110, and Howard Sprecher, Ohio State University, Columbus, OH.

Platelets preferentially incorporate potential eicosanoid precursors into phospholipids, thereby preventing their metabolism by

unstimulated cells. A correlation is found between uptake and esterification of particular fatty acids and their ability to serve as substrates for arachidonoyl-CoA synthetase. $HSDM_1C_1$ mouse fibrosarcoma cells also exhibit high affinity uptake of eicosanoid precursors. A mutant line of $HSDM_1C_1$ cells, EPU-1, has been selected for its inability to take up arachidonate at low concentrations. This EPU-1 line lacks arachidonoyl-CoA synthetase. Bradykinin-induced arachidonate release and PGE_2 synthesis are decreased in EPU-1 cells, indicating that arachidonoyl-CoA synthetase is required for normal, agonist-induced eicosanoid synthesis. The substrate specificity of arachidonoyl-CoA synthetase in platelet microsomes was examined by measuring the inhibition of arachidonoyl-CoA synthesis by various synthetic, polyenoic fatty acids with varying chain length and double-bond position. The chain-length specificity of the synthetase for Δ8,11,14-trienoic fatty acids as determined by apparent k_i values is $C_{19} = C_{20} > C_{18} >> C_{21} > C_{22}$. For positional isomers of arachidonate (20:4Δ5,8,11,14), 20:4Δ6,8,12,15 and 20:4Δ7,10,13,16 have equal apparent k_i values, however V_{max} for arachidonate is greater than that for 20:4Δ6,9,12,15. 20:4Δ4,7,10,13 has a very high k_m value and low V_{max}. The enzyme apparently counts double-bond positions from the carboxyl terminus. Several ω6,9,12 fatty acids are ineffective as inhibitors (18:3Δ6,9,12, 19:4Δ4,7,10,13, 21:3Δ9,12,15), whereas all methylene-interrupted tri- and tetraenoic fatty acids, which contain Δ8 and Δ11 double bonds, are potent inhibitors. Of the many possible double bond positions, the Δ11 double bond seems to be best associated with optimal activity: 20:3Δ5,11,14 has a lower k_i and k_m than 20:3Δ5,8,14. A substituted fatty acid, 13-Methyl-20:3Δ8,11,14 shows poor inhibitory activity.

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INCORPORATION AND METABOLISM OF LONG-CHAIN POLYENOIC FATTY ACIDS BY PLATELETS. Howard W. Sprecher, Ohio State University, Department of Physiological Chemistry, 5170 Graves Hall, 333 W. 10th Ave., Columbus, OH 43210.

Washed human platelets incorporate 18:3(n-6), 18:4(n-3), 20:3(n-9), 20:4(n-6) and 20:5(n-3) into PE, PC, PS and PI. Each substrate is also chain elongated with subsequent incorporation of the chain-elongated products into phospholipids. The specific activity in individual phospholipids was different for various substrates and chain elongated products. Rate studies with 20:3(n-9), 20:4(n-6), 20:5(n-3), 22:4(n-6), 22:5(n-3) and 22:6(n-3) define some of the structural features required to incorporate these substrates with 20:4(n-6) shows that each substrate is incorporated into platelet phospholipids in the presence of substrate saturating levels of 20:4(n-6). Washed human platelets metabolize 20:6(n-3) into 11- and 14-hydroxydocosahexaenoic acids by indomethacin insensitive enzyme(s). Adrenic acid [22:4(n-6)] is converted to dihomo-TXB_2, 14-hydroxynonadecatrienoic acid and 14-hydroxydocosatetraenoic acid.

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THE REGULATION OF ARACHIDONATE RELEASE AND HUMAN PARTURITION. John M. Johnston, Department of Biochemistry, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235.

That prostanoids are involved in the initiation of human parturition has been established. Previously, we demonstrated an increase in the amounts of prostanoids and arachidonate in amniotic fluid during labor. We demonstrated that amnion tissue is a major source of arachidonate and contains prostaglandin synthase activity. Phosphatidylethanolamine and phosphatidylinositol of amnion tissue have been identified as the source of arachidonate during early labor. Phospholipases A_2 and C, as well as di- and monoacylglycerol lipase activities have been demonstrated in amnion tissue. The phosphilipases, A_2 and C are relatively specific for phosphatidylethanolamine and phosphatidylinositol, respectively, and both require Ca^{2+}. Ca^{2+} also inhibits diacylglycerol kinase. Based on these observations, we have suggested that Ca^{2+} may regulate the liberation of arachidonate for prostanoid formation in amnion. PAF (Platelet-activating factor, 1-0-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) induces a rapid increase in cytosolic Ca^{2+} in several cell types. PAF was detected in the lipid extracts of some samples of amniotic fluid obtained from women in labor, but it was undetectable in samples of amniotic fluid obtained before the onset of labor. The addition of PAF to amnion tissue increased prostanoid formation. The relationships of these observations to the initiation of parturition will be discussed.

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INTERRELATIONSHIPS IN THE METABOLISM OF PLATELET ACTIVATING FACTOR AND ARACHIDONIC ACID IN NEUTROPHILS. Robert L. Wykle, Department of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Studies indicate that neutrophils, as other cells, synthesize platelet activating factor (PAF) from cellular 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (1-alkyl-2-acyl-GPC) by deacylation-acetylation reactions. The cells inactivate exogenously added PAF by removing the acetyl moiety and replacing it with a fatty acyl group. Several observations in our laboratory suggest that 1-*O*-alkyl-2-arachidonoyl-GPC can serve as a precursor of both PAF and bioactive arachidonate metabolites, which may then act synergistically to elicit responses. Human neutrophils contain elevated levels of 1-*O*-alkyl-2-acyl-GPC (comprising 45% of the choline-linked phosphoglycerides) that is enriched in arachidonate. Labeled arachidonate is incorporated into both the diacyl- and 1-alkyl-2-acyl species; stimulation by Ca^{2+} ionophore leads to release from both species and simultaneous formation of PAF. Lyso-PAF is selectively acylated by arachidonate in human neutrophils. The response of neutrophils can be partially blocked by lipoxygenase inhibitors; furthermore, exogenous 5-HETE potentiates the degranulating activity of PAF. The findings suggest a close relationship exists between PAF and arachidonate metabolism.

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THE EFFECT OF EICOSAPENTAENOIC ACID ON THE LEUKOTRIENE B PATHWAY IN HUMAN NEUTROPHILS. Stephen M. Prescott, Nora Eccles Harrison Cardiovascular Research & Training Institute, Nora Eccles Harrison Building, University of Utah, Salt Lake City, UT 84112.

Interest has resurfaced about the effect of dietary fatty acids on disease since studies in humans and animals have shown that a diet rich in fish oil protects against cardiovascular and other diseases. Recent investigations of the mechanism of this effect have focused on the n-3 fatty acids (particularly 20:5 and 22:6) in fish oils and their effects on the metabolism of 20:4 (n-6). In particular, 20:5 and 22:6 inhibit thromboxane production from 20:4 in platelets. We have examined the effect of 20:5 on the metabolism of 20:4 by human neutrophils (PMN) because they have been increasingly implicated as mediators of cardiovascular disease. Leukotriene B_4, a potent chemotoxin and agonist for increased vascular permeability, is the major 20:4-metabolite in PMN. The uptake of 20:5 is similar to that of 20:4 (5.0 nmol of 20:5 vs 5.2 nmol of 20:4 per 10^7 PMN in 5 min). In response to stimulation with A23187, PMN release 3.0% of their 20:5 and 3.1% of 20:4. However, the subsequent metabolism is not the same. In the presence of 20:5, PMN produce 3 new compounds, identified as LTB_5 and its nonenzymatically formed isomers. The proportion of the total products accounted for by the isomers is higher in the 20:5-derived compounds than the 20:4-derived compounds. Further, 20:5 is a less effective substrate for LTB production than 20:4 over concentrations from 5–50 μM: e.g., 20 μM 20:5–95 ng $LTB_5/10^7$ PMN, 20 μM 20:4–400 ng LTB_4. However, 20:5 is an effective inhibitor because LTB_4 synthesis decreases by 70% when 20:5 and 20:4 are present in equimolar concentrations. These, and other, observations suggest that 20:5 is a good substrate for the initial lipoxygenase reaction and the subsequent formation of LTA_5, but is a relatively poor substrate for LTA hydrolase and inhibits the pathway at this point. We tested purified LTB_5 in a bioassay and find it to be 10-fold less potent than LTB_4. Thus, the incorporation of 20:5 (n-3) into PMN results in a net decrease in LTB production and an even greater loss in biological activity. These findings indicate that a portion of the beneficial effect of diets rich in fish oil on cardiovascular disease may be caused by the effect of 20:5 on 20:4 metabolism in PMN.

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RETINOIC ACID AND PROSTAGLANDIN E_2 PRODUCTION BY HUMAN SKIN FIBROBLASTS. R. Batres and J. Dupont, Food and Nutrition Department, Iowa State University, Ames, IA 50011.

Hyperlipidemia and alterations in lipid metabolism, as well as therapeutic dermatological effects, are produced by the intake of retinoic acid. The purpose of this study was to determine whether retinol acetate (RAc), all-*trans* retinoic acid (tRA), or 13-*cis* retinoic acid (13-cRA) induce changes in prostaglandin E_2 (PGE_2) synthesis by human newborn skin fibroblasts. Fibroblasts (between 40 and 50 passages) were grown to confluence and then incubated for 72 hours with either 0.1, 0.6 or 3.0 μg per mL of culture medium (minimum essential media with 10% fetal bovine serum) of the 3 retinoids. The culture medium was then collected, and the fibroblasts were stimulated with 0.5 unit of thrombin for 5 min. PGE_2 levels were measured in the culture medium and in the medium collected after stimulation with thrombin. PGE_2 production by these cells was compared with PGE_2 production by fibroblasts in which the tissue culture medium was not supplemented with any of the 3 retinoids. After incubation with 13-cRA thrombin stimulated fibroblasts had markedly reduced synthesis of PGE_2 (8% of control) at the lowest concentration. RAc and tRA had no effect. PGE_2 concentration in the medium after 3 days of incubation was increased by both RAc and tRA and severely decreased by 13-cRA. These results suggest that some of the effects of retinoids may be mediated by prostaglandin metabolism in the cells.

Session U Surfactants and Detergents II: Performance and Evaluation Tuesday p.m.

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EFFECT OF SURFACTANT STRUCTURE ON STABILITY OF ENZYMES FORMULATED INTO LAUNDRY LIQUIDS. L. Kravetz and Kathryn F. Guin, Shell Development Company, P.O. Box 1380, Houston, TX 77001.

The effect of surfactant structure on the stability of enzymes in heavy-duty laundry liquids was investigated. Both nonionic ethoxylates and anionic surfactants having varying hydrophobic and hydrophilic types and chain lengths were included. The results showed enzymes were considerably more stable when formulated into laundry liquids containing nonionic ethoxylates and alcohol ethoxysulfates than when formulated with surfactants containing sulfonate groups, e.g., linear alkylbenzene sulfonates and paraffin sulfonates. For alcohol ethoxylates, no significant effect on the stability of enzymes was observed by varying the alkyl chain length. However, enzyme stability appears to improve somewhat with increasing polyoxyethylene chain length.

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USE OF ENZYMES TO IMPROVE WASH PERFORMANCE AT LOW TEMPERATURE. Daniel Kochavi and J. M. Monnier, Novo Laboratories, 59 Danbury Road, Wilton, CT.

Trends toward energy savings and use of synthetic fabrics have resulted in the increase use of low-temperature wash settings. Often, lowering the temperature results in the loss of detergency by heavy-duty powders or liquids. Our studies indicate that the addition of enzymes to these detergents can compensate for the loss in their cold-wash efficacy. Three NOVO enzymes, Alcalase, Esperase and Savinase were evaluated in several leading US heavy-duty detergents. The wash performance at 70 F, with and without enzymes, was compared with that at 120 F (49 C) without enzymes. For these tests, EMPA116, egg on cotton and grass stains were used. With no added enzymes, all but 1 product showed a decrease in detergency at 70 F, irrespective of the stain used. In the case of EMPA116 and egg stains, the addition of enzyme significantly increased the detergency to levels equal to or above those found at 120 F. To a lesser degree, similar effects were found with grass stains. For each heavy-duty product, the magnitude of the increase followed the sequence Alcalase ≥ Savinase > Esperase. The addition of enzymes to heavy-duty powders or liquids can compensate for the loss in cold-water wash detergency on selected stains. The extent of this compensation varies with detergent products as well as with the enzyme used. Wash tests with other fabrics and other NOVO enzymes are planned to further test these observations.

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EVALUATION OF MULTIFUNCTIONAL BENEFITS OF POLYACRYLATE POLYMERS IN DETERGENT COMPOSITIONS. M. K. Nagarajan, B.F. Goodrich (Chemical Group), Technical Center, P.O. Box 122, Avon Lake, OH 44012.

Alkali metal salts of polyacrylic acid polymers are water-soluble, multifunctional polyelectrolytes that exhibit a variety of solution properties useful in powder or liquid laundry and dishwashing detergents—both in consumer and institutional cleaning products. This paper describes a number of studies carried out to identify the multifunctionality of polyacrylate polymers under simulated detergent use conditions. Solution properties of several commercially available polyacrylate polymers, with weight average molecular weight ranging from ca. 2,500 to 250,000 are presented, e.g., adsorption onto model particulate soil materials and fabrics, particulate soil and lime-soap dispersancy, sequestration of calcium, magnesium and ferric ions, calcium carbonate precipitation inhibition and detergency. Where appropriate, these solution properties are compared with those of commonly used nonpolymeric detergent ingredients. The relationship between polyacrylates' molecular weight and the above-mentioned functions in detergent systems is also briefly discussed.

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ANTIFUNGAL PROPERTIES OF 3-*n*-ALKYN-1-OLS. Herman Gershon, Boyce Thompson Institute, Tower Road, Ithaca, NY 14853.

Twelve 3-*n*-alkynols (C_4–C_{12}, C_{14}, C_{16} and C_{18}) were tested against *Aspergillus oryzae, Aspergillus niger, Tichoderma viride* and *Myrothecium verrucaria* in Sabouraud dextrose agar at pH 5.6 and 7.0. Toxicity to *Candida albicans, Candida tropicalis, Trichophyton mentagrophytes* and *Mucor mucedo* was determined in the same medium at pH 5.6 and 7.0 in the absence and presence of 10% beef serum. Fungitoxicity was strongly influenced by chain length, slightly by pH of the medium and significantly by the presence of beef serum. 3-*n*-Decyn-1-ol, 3-*n*-undecyn-1-ol and 3-*n*-dodecyn-1-ol were the most active members of the series. Comparisons will be made between this series of 3-alkyn-1-ols and a comparable series of 2-alkyn-1-ols. Synergism between the alkynols and ketaconozole was observed.

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HOW CAPILLARY PRESSURE AFFECTS THE PRODUCTION WELL. Erle C. Donaldson, University of Oklahoma, Petroleum Engineering Department, University of Oklahoma, 865 Asp Avenue, Norman, OK 73019.

The capillary pressure between water and oil is a function of the fluid saturations of the porous medium. Capillary pressure in the vicinity of a production well plays a very important role in flow of the 2 fluids into the well. Assuming water is the wetting phase, the pressure of he aqueous phase must be continuous within the porous system and in the well. Because the capillary pressure in the well does not exist (is zero), water will not flow into the well until a condition of zero capillary pressure is obtained within the formation in the immediate vicinity of the well. Therefore, a newly drilled well initially produces only oil until the capillary pressure in the sand reduces to zero. The inhibition capillary pressure curve applies because the water saturation is increasing as production proceeds. Water is then produced in greater quantities as the water saturation, at zero capillary pressure, moves to the water saturation at residual oil. When this point is reached production of oil stops. Capillary pressure curves are presented to show these effects. Data are also presented to show the effect of the movement of fine particles toward the wellbore, which decreases the pore sizes and adversely affects the capillary phenomena at the wellbore.

Meetings

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ASPHALTENE AND WATER ADSORPTION ON CLAYS. James L. McAtee, Jr. and Kim Dean, Baylor University, Chemistry Department, Waco, TX 76798.

Asphaltene adsorption onto kaolin and smectite surfaces varies, depending on the amount of water present in the system, temperature, sample size and the type of clay used. Adsorption experiments were carried out by mixing clays and various concentrations of asphaltene dissolved in toluene. The samples were shaken for 24 hr; then the concentration of asphaltene remaining in the solvent was determined. From this data, various isotherms were plotted to describe the adsorption. Cations-exchanged (Na^+, K^+, Ca^{++}, Mg^{++}) kaolins with 8% preadsorbed water showed no decrease in asphaltene adsorption compared with dried clays at room temperature. Increasing the water to 30% lowered the adsorption to zero. Higher temperatures increased the rate and amount adsorbed. Smectite–asphaltene/water adsorption was also studied.

Session V Trends in Industrial Usage for Vegetable Oils Tuesday p.m.

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SULFURIZED VEGETABLE OIL PRODUCTS AS LUBRICANT ADDITIVES. Karl Kammann and Astrid Phillips, Keil Chemical Division, Ferro Corporation, 3000 Sheffield Ave., Hammond, IN 46320.

Sulfurized products based on hog grease and its derivatives have extensive commercial use as additives for metalworking and industrial oils, but only small quantities of vegetable oils find such application in North America. The products were made by sulfurization of soybean, sunflower, cottonseed, high erucic rapeseed, canola, limnanthes (meadowfoam) and prime lard oil. Unlike products from te wax ester jojoba oil, the sulfurized vegetable triglycerides had physical properties generally undesirable for lubricant additives, but when the oils were sulfurized in the presence of methyl lardate, the products were shown to have potential practical application. High-sulfur (active) products were made using a 50:50 ratio of triglyceride to methyl lardate, and low-sulfur (inactive) products were made using a 70:30 ratio. Compared with other high-sulfur vegetable oil products, limnanthes products showed outstanding solubility in high viscosity index paraffinic oil. For solutions, measurements of extreme pressure, friction and wear were compared, and the limnanthes-containing products generally gave the best performance. While the results were promising for this oil, which is only in its early stage of commercial development, the other vegetable oils also have potential, depending on cost and applications. However, overall competition with the well-established and usually lower-cost products from hog greases would appear to be difficult.

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USES OF SOYBEAN OIL IN THE APPLICATION OF HERBICIDES. George Kapusta, Department of Plant and Soil Science, Southern Illinois University, Carbondale, IL 62901.

Soil- and foliar-applied herbicides are used on ca. 200 million ha in the US. Worldwide, the total is ca. 500 million ha. Before application, herbicides are diluted in 150 to 200 L/ha diluent if applied with ground equipment and in 25–40 L/ha if aerially applied. Currently, water is used to dilute the herbicides in essentially all instances. In additon to water, many foliar herbicides require an enhancing agent such as a phytobland petroleum oil at a volume of 2–3 L/ha for maximum herbicide efficacy. The use of vegetable oils to dilute herbicides has been too expensive in the past because the hydraulic sprayers used require a minimum of about 50 L/ha for satisfactory performance. The recent commercialization of ultra-low volume (spinning cone) spray equipment allows the application of most herbicides in volumes as low as 1–2 L/ha, making the use of vegetable oil as a herbicide carrier realistic. Our research has evaluated the use of soybean oil as a substitute for water as a carrier for herbicide applications and as an enhancing agent in place of petroleum oil, which is currently being used. My results indicate that with solution or emulsifiable concentrate formulations, soybean oil at volumes as low as 2 L/ha applied with ultra-low volume sprayers substitutes for 200 L/ha water applied with hydraulic sprayers without a loss of herbicidal efficacy. Several major herbicides have been evaluated. However, pure soybean oil has not been satisfactory for diluting wettable power herbicides. Using a soybean oil/water system to suspend these herbicides satisfactorily has been necessary because they are formulated to dissolve or suspend in water. The use of a soybean-oil concentrate (85% soybean oil plus 15% emulsifiers) in lieu of petroleum crop-oil concentrate (83% petroleum oil plus 17% emulsifiers) has been very promising. Initially, this use would not require the purchase of ultra-low volume equipment. Further, in essentially all of our evaluations, the soybean-oil concentrate has enhanced foliar herbicides equally to petroleum crop-oil concentrate. These results indicate that soybean oil can be used at very low volumes as a carrier for herbicides or as a substitute for petroleum oil used as an enhancing agent with foliar herbicides.

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TRENDS IN THE INDUSTRIAL USE OF VEGETABLE OILS IN COATINGS. Richard W. Fulmer, Cargill, Inc., P.O. Box 9300, Minneapolis, MN 55440.

Alkyd resins continue to be a major factor in coatings. Increased oil consumption in alkyd manufacture is not expected to be significant. Projections indicate growth in the use of total coatings at a modest 2–3%/year. The industry is facing diverse coating performance demands that will bring unusual, more costly ingredients into use, probably at the expense of traditional oil-based alkyd resins. Offsetting this decline in oil use, perhaps, will be the continuing cost advantage of the relatively low-priced vegetable oils and the general versatility of alkyd resins. An increased use of oil-based resins is expected in emulsion (latex) paint modifiers to improve adhesion and early water resistance. The coatings industry, at least in maintenance and industrial coatings, is adopting a cost/sq. ft./year economic evaluation, factoring in the useful life of the coating.

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SOYBEAN OIL: ITS SOAPSTOCKS AND PHYSICAL REFINING DISTILLATES, THE GROWING POTENTIAL FOR INDUSTRIAL USE. N. O. V. Sonntag, 306 Shadowwood Trail, Red Oak, TX 75154.

Soybean oil is the cheapest, most readily available vegetable source of high C-18 stearic acid. Although it is priced too high to complete generally with inedible tallow for bulk industrial uses, or for the manufacture of oleic acid, it is now used, and will be used increasingly in the future, for high C-18 stearic acid derivatives, particularly those that involve the manufacture of C-18-containing food additive ester derivatives, of which sodium and calcium stearoyl 2-lactylate and propylene glycol monostearate are examples. If new processing developments are successful, soybean-oil soapstocks, from the conventional refining of soybean oil, and distillates from the physical refining of this oil, will provide an economically attractive source of stearic acid for many bulk industrial uses in the future. The technical problems remaining to be solved in order to achieve this are outlined. Several potential industrial applications for soy fatty acids, hydrogenated soy fatty acids, soy monoglycerides and for soybean oil itself are proposed.

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CHEMICAL EPOXIDATION OF *Vernonia galamensis* OIL, A NATURAL EPOXY SEED OIL. K. D. Carlson and S. P. Chang, Northern Regional Research Center, 1815 N. University Street, Peoria, IL 61604.

Referred to collectively as epoxidized esters (EE), three basic groups of epoxidized products are made from vegetable oils: epoxidized triglyceride oils, mixed epoxy fatty esters (e.g., tallates), and specific epoxy fatty esters. Their importance as plasticizers in flexible PVC resins stems from heat and light stability imparted to the finished products. Since 1963, production of all EE has ranged from 60 to 150 million lb annually, a steady 7% of the 1 to 2 billion

lb of annual plasticizer production. Growth rates in production averaged 0.3% for all plasticizers, 3.8% for all EE, and 5.0% for epoxidized soybean oil (ESBO). ESBO accounts for 70–76% of the total EE production (1963–81). The higher oxirane oxygen contents (7–9%) molecular weights (ca. 1000), and viscosities of epoxidized triglycerides, compared with corresponding lower values for the epoxy fatty esters (4–5% oxirane, molecular weights ca. 000–400), provide a useful range of properties for specific applications. The natural liquid epoxy oil from *Vernonia galamensis* seed, with oxirane value (4.2) and viscosity (100 cps) similar to the epoxy fatty esters but with a molecular weight similar to epoxidized vegetable oils, combines some of the properties of both commercial types. Chemical epoxidation of *Vernonia* oil raises the oxirane content to 8.2, intermediate between ESBO and epoxidized linseed oil (ELSO), while consuming less of the costly epoxidizing reagents. Epoxidation with m-chloroberbenzoic acid proceeds step by step through partially epoxidized products, which are converted to the final product. Because the major fatty acids of *Vernonia* oil are 12,13-epoxy-9,10-octadecenoic (75%) and linoleic (13%), on epoxidation nearly 90% of the product fatty acids will be specifically epoxidized at the 9,10- and 12,13-positions and the principal products will have 5 to 6 epoxy units per triglyceride molecule. The resulting mixture of products is distinctly different from commercial samples of ESBO and ELSO.

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THERMOCHEMICAL APPLICATIONS FOR FATS AND OILS. E. S. Lipinsky, D. Anson and J. R. Longanbach, Battelle, Columbus Laboratories, 505 King Avenue, Columbus, OH 43201.

Fats and oils are well established in food, surfactant, and coatings applications. Historically, fats and oils were employed to provide lighting and warmth. The increasing production of fats and oils and the increasing uncertainties regarding the reliability of petroleum resources make reconsidering thermochemical applications of fats and oils desirable. Pyrolysis of fatty acids at temperatures exceeding 500 C yields a mixture of gaseous and liquid products. The gaseous products include ethylene, but little propylene. The liquid product is primarily a mixture of substituted styrenes. The implications of these results will be discussed. Conventional kerosene space heaters are designed to make use of the vaporization behavior of this fuel. The changes in heater design to make use of fats and oils will be discussed. The design of effective combustors for fats and oils must take into account pyrolytic degradation of this class of compounds.

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RICINOLEATE-BASED POLYURETHANES IN TELECOMMUNICATION APPLICATIONS. F. Naughton, Melvin Brauer and William Downey, CasChem, Inc., 40 Avenue A, Bayonne, NJ 07002.

Ricinoleates are used in polyurethanes because of the versatility they present to the formulator. In the telecommunications industry, castor oil-based polyurethanes are employed in cable reclamation and encapsulation as well as for elastomeric potting compounds for transformers, terminal blocks and relays. Compared with polyester- and polyether-based polyurethanes, the ricinoleate-based polyurethanes demonstrate superior electrical properties and hydrolytic stability, caused by the fewer number of ester groups and the absence of ether linkages, both being polar groups that can be hydrolyzed. These superior characteristics are especially desirable in telecommunications and are apparent in the data presented. In addition to these benefits, other desirable properties such as a low stress and strain relationship, low curing exotherms and low shrinkage are obtained using ricinoleate-based polyurethanes. These properties are especially important when encapsulating pressure- or temperature-sensitive equipment. The property relationships between ricinoleate-based and polyester- and polyether-based polyurethanes, as well as their use in the potting and encapsulation of telecommunications equipment will be discussed in this paper.

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RICINOLEATE-BASED POLYURETHANES IN BIOMEDICAL APPLICATIONS. Jack Chu, M. Brauer and F. Naughton, CasChem, Inc., 40 Avenue A, Bayonne, NJ 07002.

Ricinoleates are employed in polyurethane systems serving the biomedical field in such critical areas as blood dialysis and filtration. They are especially useful here because of their superior blood compatibility, hydrolytic stability and moisture insensitivity. Compared with polyether- and polyester-based polyurethanes, the relationships of solubility parameters to hydrolytic stability and moisture sensitivity may explain the superiority of ricinoleate polyurethanes. Selected Biothane^{TM} polyurethane systems designed for use in the centrifugal potting of hollow-fiber artificial kidneys provide excellent adhesion to fibers and to the plastic casing materials. In addition, the ricinoleate-based systems have excellent processing and cutting characteristics. These systems also provide excellent resistance to degradation during steam sterilization, and they exhibit low swelling or leaching in aqueous solutions, such as blood. Therefore, well-designed, ricinoleate-based polyurethane systems pass critical blood cytotoxicity and hemolysis tests.

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STABILITY STUDIES ON SUNFLOWER-SEED OIL METHYL AND ETHYL FATTY ACID ESTERS. L. M. du Plessis, J. B. M. de Villiers and W. H. van der Walt, National Food Research Institute, C.S.I.R., P.O. Box 395, Pretoria, 0001, Republic of South Africa.

Fatty acid esters were prepared for long-term fuel tests on diesel engines and, as linoleic acid (the main component of sunflower-seed oil) is relatively unstable, more information on the storage requirements and stability characteristics of the esters was needed. Fatty acid ester samples were stored at 3 temperature levels (20 C, 30 C and >50 C) for a 90-day period and samples were removed at regular intervals for chemical and physical analysis. The influence of temperature, air, light, soft steel and a synthetic antioxidant was evaluated by comparing the acid, peroxide, anisidin, tocopherol and ultraviolet absorption values as well as the viscosity and induction periods. Exposure of esters to air (oxygen), especially at higher temperature levels, was harmful to the esters. Exposure of esters to daylight was less harmful and the deleterious effect was only noticeable at the highest temperature level. Soft steel affected the esters very little and in general we found that methyl esters performed better than ethyl esters during the storage test. The following practical guidelines for storage of fatty acid esters fuels are: (a) use airtight containers filled to the top; (b) storage temperature should be below 30 C; (c) clean, rust-free, soft steel containers may be used.

Session W Nutritional Aspects of Fatty Acids Tuesday p.m.

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NUTRITION IN RELATION TO GAS LIQUID CHROMATOGRAPHIC PROFILES OF PLASMA TOTAL LIPIDS. A. Kuksis, J. J. Myher, K. Geher, W. C. Breckenridge, T. Feather, V. McGuire and J. A. Little, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.

Fasting plasma total lipid profiles were determined by high-temperature gas liquid chromatography on a total of 1,100 free living urban subjects, ages 20–70 years, from the Lipid Research Clinic Population Study. Quantitative estimates for individual molecular species, lipid classes and lipid class ratios were correlated with a total of 31 dietary components, including various micronutrients, to give appropriate Spearman coefficients (r_S) and tests of significance (P) for groups of 775 males, 250 females and 80 females taking gonadal hormones. The qualitative and quantitative dietary intakes of the various nutrients were derived from a 24-hr dietary recall. The most significant correlations ranging from $r_S \pm 0.2–0.4$; $P<0.001–0.005$ were between the intake of total fat, individual saturated and unsaturated fats, starch and alcohol in relation to the ratios of C_{54}/C_{50} triacylglycerols, the C_{36}/C_{38} phosphatidylcholines and sphingomyelin/phosphatidylcholine. The latter ratio also was

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found to correlate significantly with the intake of various micronutrients. Dietary cholesterol showed no significant correlation with any of the parameters of the total lipid profile. The data were highly consistent between the 2 groups of females, but differed greatly between males and females. The number of dietary components showing significant correlation with the total lipid profiles was greatest for the males and smallest for the females on hormones reflecting to some extent the size of the test groups. The high correlation between the dietary intakes and the various ratios of the lipids in the total lipid profiles appears to reflect both the effect of nutrition on the relative levels of plasma lipoprotein classes and changes in their lipid composition. A highly significant parallel correlation between the levels of specific dietary ingredients and the levels of individual lipoprotein classes and the corresponding lipid class ratios in the total lipid profiles was found. We conclude that a determination of plasma total lipid profiles can provide significant information about the relative intake of different dietary fats, carbohydrates and alcohol and about the relative proportions of high and low density lipoproteins, and has the potential of indicating the subjects' adherence to therapeutic diets.

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BIOCHEMICAL PATTERNS OF EFA DEPLATION AND REPLETION. Eldon G. Hill, Bruce A. Svingen and Ralph T. Holman, The Hormel Institute, University of Minnesota, 801 – 16th Avenue, N.E., Austin, MN 55912.

The changes that occur in the patterns of polyunsaturated fatty acids (PUFA) of tissue as a consequence of onset or correction of essential fatty acid deficiency (EFAD) are well known. The effect of duration of EFAD or of the duration of previous linoleic acid intake on these changes is not known. Therefore, a nutritional experiment was performed to describe the onset of EFAD initiated at different ages in rats previously fed an EFA-adequate diet. Conversely, the effects of repletion of the ω6 fatty acids by dietary linoleate after varying lengths of time of EFAD were also described. The analysis of the fatty acids of liver phospholipids (PL) were made at appropriate intervals throughout the experiment to describe the depletion and repletion phenomena. In the initial stages of this study, the curves describing repletion of liver 20:4ω6 seemed close to parallel after lengths of 4, 8 and 12 weeks of EFAD. The depletion of 20:4ω6 after 4, 8 and 12 weeks of linoleate supplementation yielded curves that were similar but not parallel. Very similar but opposite phenomena were observed for 20:3ω9. Analyses of liver subcellular fractions corroborated findings based on whole liver.

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EFFECT OF THE CHEMICAL FORM OR THE MODE OF ADMINISTRATION OF ESSENTIAL FATTY ACIDS ON THEIR FURTHER METABOLISM. Armand Christophe, Eldon G. Hill and Ralph T. Holman, The Hormel Institute, University of Minnesota, 801 – 16th Avenue, N.E., Austin, MN 55912.

This study was intended to determine if the chemical forms of the compounds in which dietary fatty acids are fed or the route of administration may affect the kind of tissue that takes them up, and thus their further metabolism. Essential fatty acid deficient (EFAD) male rats were given a fat-free diet supplemented with a source of essential fatty acids. These diets were either fed as sunflower-seed oil or as molecular-distilled isomeric monoglycerides from this oil at a level of ca. half of their essential fatty acid requirement. In another group of EFAD rats, the monoglycerides were administered through the rectum. The effect of the 3 different treatments on the major blood and liver lipid levels and their fatty acid composition was determined. The results and their possible implications for clinical nutrition will be discussed.

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GEOMETRICAL AND POSITIONAL FATTY ACIDS ISOMERS IN THE HUMAN DIET. M. A. (Vic) Amer, Dairy Bureau of Canada and McDonald College of McGill University, 1981 McGill College Avenue, Suite 1330, Montreal, Quebec H3A 2X9, Canada.

In the processing of vegetable fats and oils to produce margarines and shortenings, a significant amount of geometric isomers, *trans* fatty acids are formed as a result of commercial hydrogenation. The number and concentration of the unsaturated geometrical and positional octadecenoic *trans* fatty acid isomers naturally occurring in milk fat were determined and compared with *trans* isomers resulting from catalytic hydrogenation of vegetable oils. To determine geometric isomerization and double-bond location, argentation thin layer and gas liquid chromatography were employed in conjunction with ozonolysis to split the fatty acid at the position of unsaturation. Results obtained in this study were used to survey the per capita consumption of various *trans* monoenes. The effect of the natural (rumen hydrogenated) and the unnatural (catalyst-induced hydrogenation) dietary *trans* octadecenoate isomer is reviewed.

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IN VIVO DISTRIBUTION OF *trans*- AND *cis*-10-OCTADECENOIC ACID ISOMERS IN HUMAN PLASMA LIPIDS. Edward A. Emken, W. K. Rohwedder, R. O. Adlof and W. J. DeJarlais, Northern Regional Research Center, 1815 N. University Street, Peoria, IL, and R. M. Gulley, St. Francis Medical Center, Peoria, IL 61637.

Triglycerides containing deuterium-labeled *trans*-10 and *cis*-10-octadecenoic acid (10t-18:1, 10c-18:1) plus the triglyceride of deuterated *cis*-9-octadecenoic acid (9c-18:1) were fed as a mixture to 2 young adult male subjects. Analysis by mass spectroscopy (MS) of the labeled fats in blood samples collected periodically for 48 hours allowed the uptake, distribution and turnover of both 10-octadecenoic acid isomers to be directly compared with 9c-18:1. Ca. equal amounts of all 3 deuterated fats were incorporated into the chylomicron triglycerides and into most plasma and lipoprotein phospholipids. Very low, low and high density lipoprotein triglyceride samples contained 7–25% less 10t- and 10c-18:1 relative to 9c-18:1 than were present in the mixture fed and in chylomicron triglyceride samples. These data indicate that use for β-oxidation or deposition into tissue is more rapid for 10t- and 10c-18:1 than for 9c-18:1. In contrast, more 10c-18:1 and less 10t-18:1 compared with 9c-18:1 were incorporated into plasma phosphatidylcholine. Two times more 10c-18:1 than 10t-18:1 was present in both the 1-acyl and 2-acyl positions of phosphatidylcholine (PC) and 3–5 times more 10c- and 10t-18:1 were incorporated into 1-acyl PC compared to 9c-18:1. Very strong discrimination against esterification of cholesterol occurred. Conversion into 16:1 and 20:1 was substantially greater for the 10c- and 10t-18:1 isomers than for 9c-18:1.

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EFFECTS OF DIETARY ISOMERIC FATTY ACIDS ON PLATELET MEMBRANE COMPOSITION AND FUNCTION AND HEPATIC FATTY ACID DESATURATION IN THE RAT. Rolf Blomstrand, Leslie Sisfontes, Ulf Diczfalusy and Bengt Lindbäck, Department of Clinical Chemistry, Karolinska Institutet, Huddinge University Hospital, S-141 86 Huddinge, Sweden, and Lennart Svensson, KabiWitrum AB, Stockholm, Sweden.

In previous studies, we investigated the influence of partially hydrogenated marine and vegetable oils on rat-heart mitochondrial function and on fatty acid desaturase activities in liver microsomes. In the present study, we have extended the study to include the influence of isomeric fatty acids on platelet membrane composition and function. Rats were fed partially hydrogenated Lobra and partially hydrogenated herring oil, with and without a linoleic acid supplement. A reference group was fed olive oil supplemented with linoleic acid. Δ6- and Δ5-Desaturase activities were determined in the different dietary groups and the liver microsomes were analyzed for their fatty acid composition. Platelet lipid extracts were resolved into individual phospholipid classes by high pressure liquid chromatography and the fatty acid composition was determined in each class. Washed platelets were stimulated with thrombin and the reaction mixture was analyzed for 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT). Aortas were incubated to study the endogenous production of 6-keto-prostaglandin $F_{1\alpha}$. The results from this investigation will be presented and the influence of dietary fat on fatty acid desaturation and further metabolism of arachidonic acid into oxygenated metabolites will be discussed.

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INFLUENCE OF REDUCED FOOD INTAKE ON POLYUNSATURATED FATTY ACID METABOLISM IN ZINC-DEFICIENT RATS. Susan B. Johnson and Ralph T. Holman, The Hormel Institute, University of Minnesota, 801 – 16th Avenue, N.E., Austin, MN 55912.

The influence of reduced food intake on the metabolism of liver phospholipids in zinc-deficient rats was determined. Weanling male Long-Evans rats were fed, ad libitum, a zinc-deficient (2 μg Zn/g diet) and a zinc-adequate (20 μg Zn/g diet) diets for 21 days. A pair-fed group was fed a zinc-adequate diet but restricted to the amount consumed by zinc-deficient rats. Growth and food intake were significantly depressed in zinc-deficient and pair-fed rats. Zinc content in serum was significantly reduced in zinc-deficient rats. Fatty acid composition of liver phospholipids was determined. Zinc-deficient and pair-fed rats displayed significantly increased levels of 18:2ω6 and 20:3ω6 and decreased levels of 20:4ω6 and 22:5ω6. Both zinc-deficient and pair-fed rats displayed increased products of Δ6 and Δ9 desaturation and decreased products of Δ5 and Δ4 desaturation. Zinc-deficient and pair-fed rats displayed significant increases in C_{20} elongation products, but C_{22} elongation products were not significantly different among the 3 dietary groups. Levels of total ω6 acids were not significantly different among the 3 dietary groups, however, zinc-deficient and pair-fed rats displayed significantly decreased levels of ω6 metabolites. Zinc-deficient rats showed significantly increased levels of total ω3 and ω3 metabolites. Zinc-deficient and pair-fed rats showed significant increases in ω9 acids. This study does not indicate that zinc affects the Δ6 desaturase in the metabolism of essential fatty acids. The aberrations found in zinc deficiency are probably caused by the accompanying protein-energy deficiencies.

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EFFECT OF ETHYL LINOLENATE AND LINOLEATE ADMINISTRATION ON LIVER PHOSPHOLIPID FATTY ACID COMPOSITION IN EFA-DEFICIENT RATS. Y. S. Huang, D. F. Horrobin and M. S. Manku, Efamol Research Institute, P.O. Box 818, Kentville, Nova Scotia, Canada.

The object of this study was to examine whether sex affects the metabolism of linoleic and α-lenolenic acids in EFA-deficient animals. Weanling male and female rats were fed ad libitum on a fat-free diet for 6 weeks. The status of EFA deficiency was established by a significant increase in the level of 20:3n9 in plasma phospholipids. After EFA deficiency was induced, animals of each sex were separated into 2 groups and received intraperitoneal injections of 200 μl. pure ethyl linoleate and ethyl linolenate every second day for 10 days. Fatty acid profiles of liver phospholipids were then examined. The administration of linoleate causes in increase in n-6 fatty acids. The levels of 18:2n6 and 20:4n6 increased, reaching nearly 70% of normal levels. The levels of 20:3n6 and 22:5n6 also increased. Ethyl linolenate administration, on the other hand, raised the levels of n-3 fatty acids, leading to increases of 22:5n3 and 22:6n3 and a striking accumulaton of 20:5n3 in liver phospholipids. This result suggests that the step regulating the elongation of 20:5n3 to 22:5n3 was not as effective as desaturation of 18:3n3. Male EPA-deficient rats have higher proportions of 16:0 and lower level of 18:0 than females with or without treatment with EFA. Both linoleate and linolenate reduced the increased level of 20:3n9 after being administered for 10 days, but linolenate was more effective than linoleate. Male rats retained more 20:3n9 but less 20:5n3 and 22:6n3 than did females during linolenate treatment. No six differences were observed with linoleate. The regulation of the metabolism of 18:3n3 in EFA-deficient animals may be affected by sex.

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INFLUENCE OF DIETARY ELAIDIC ACID ON THE LIPID COMPOSITION AND PHYSICAL AND FUNCTIONAL PROPERTIES OF RAT MITOCHONDRIA. B. Entressangles, N. Combe and R. Wolff, Unité de Biochimie Biotechnologie ITERG, Laboratoire de Lipochimie Alimentaire, Université de Bordeaux I, Allée des Facultés, 33405 Talence Cedex, France.

For 1–7 weeks, rats were fed diets rich in elaidic acid (ca. 65% of total fatty acids) that provided various linoleic acid levels (0.3–7.0% of total fatty acids). We have shown that the extent of dietary elaidic acid incorporation into lipids of mitochondrial membranes decreased in the following order: heart > liver > kidney. Considering a given organ, the percentage of incorporation of dietary elaidic acid decreased from phosphatidylethanolamine to phosphatidylinositol and phosphatidylcholine. In all organs, whatever the dietary linoleic and elaidic acids levels, elaidic acid was not significantly incorporated into mitochondrial cardiolipin (≤1% of total fatty acids). Furthermore, we have observed that dietary elaidic acid was incorporated, via its corresponding *trans* alcohol, into plasmalogen alk-1-enyl chains. When diets provided correct linoleic acid quantities, elaidic acid was reversibly incorporated at the expense only of saturated acyl chains or saturated alkenyl chains. When diets were marginally deficient in linoleic acid, the dietary elaidic acid was converted into 5-*cis* 9-*trans* 18:2 acid and 7-*cis* 11-*trans* 20:2 acid, which were incorporated in the place of all *cis*-polyunsaturated fatty acids, i.e., arachidonic acid. In our conditions, whatever the elaidic containing diets studied, we have never observed modifications either in mitochondrial membrane microviscosity or in ATP biosynthesis rates, compared with controls.

Session X Technical Plant Engineering Tuesday p.m.

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AN ECONOMIC ANALYSIS OF MILLING COTTONSEED WITH DIFFERENT RESIDUAL LINTERS CONTENTS. John T. Farnsworth, Lawrence A. Johnson and Edmund W. Lusas, Food Protein Research and Development Center, FM Box 183, Texas A&M University, College Station, TX-77843, and Robert J. Hron, USDA-ARS Southern Regional Research Center, New Orleans, LA.

An engineering-economic computer simultation model of cottonseed-oil milling was developed and used to estimate the profitability of alternative processes to saw delintering and single hulling of cottonseed. The model was capable of evaluating most foreseen alternatives: it predicted product yields within 1.3% of data from an operating mill and predicted average net revenues within 2.3% of those predicted by previous, less flexible economic models. Conventional mills, operating in the Southwest during 1980, were estimated to yield $39.68 average net revenue per ton of seed, based upon 600 tpd direct solvent extraction mills. Eliminating saw delintering without altering hulling-separating practices decreased average net revenue by 50%. Single hulling and separating of undelintered seed with bar-type hullers was unattractive at all prices for linters. Defibrating woolly hulls after hulling and separating undelintered seed increased average net revenues by an additional $2 per ton. However, if prices paid for linters dropped below typical 1980 prices, universal hulling or decorticating of undelintered seed became attractive, even without defibrating. Saw delintering to intermediate levels of residual linters was not attractive until prices dropped by 35% from 1980 prices. Costs for state of the art dust control reduced average net revenues by as much as $1.38 per ton in conventional mills, or as little as $0.43 per ton for some alternatives. During 1984, prices paid for all products changed drastically, thereby altering the net returns and selection of more profitable processes.

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A DIFFERENT APPROACH TO HOT DEHULLING. Hans Stricker, Buhler-Miag, Inc., 1100 Xenium Lane, Minneapolis, MN 55441.

A few years ago, the use of the hot dehulling process for the preparation of soybeans for solvent extraction was introduced to the crushing industry. The significance of this process is the savings in thermal energy of up to 50% over a conventional system. However, part of these savings had been used up by a higher consumption of electrical energy. Replacement of and changes on certain components in the process has resulted in lower electrical energy requirements without a loss of efficiency in the thermal part of the process.

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MIVAC (MICROWAVE VACUUM) PROCESSOR. Sadru Dada, World Processing Division, Continental Grain Company, 277 Park Avenue, New York, NY 10172, Hal E. Bland, Aeroglide Corporation, Raleigh, NC 27626-9990, and Howard F. McKinney, McDonnell Aircraft Company, P.O. Box 516, St. Louis, MO 63166.

Electromagnetic waves applied in a partial vacuum replace heated air in this processing system. Laboratory and prototype MIVAC (an acronym for microwave vacuum) driers have been used to dry a wide variety of agricultural and industrial products. These include such diverse products as apples, books, cotton, grapes, hops, peanuts, pine cones, ABS plastics, rice and seed corn. This paper deals with the MIVAC process and its general application with particular emphasis on processing soybeans at oil extraction plants and refineries. Experiments revealed that soybeans processed in the prototype MIVAC released their hulls more completely, more quickly, with less energy and less moisture lost. Reduction of the unwanted fiber contained in the hulls is required for continuous production of high-protein soybean meal. A soybean preparation room with a MIVAC unit could eliminate tempering bins, may alter or reduce the aspiration required and would eliminate the conditioner as now constructed and used. To verify these claims, a MIVAC soybean demonstrator drier was built under a joint venture agreement between McDonnell Douglas and Aeroglide. The unit was installed at a soybean plant operated by the World Processing Division of Continental Grain Company at Guntersville, Alabama. Tests were run throughout the summer and fall of 1983. Results of these tests will be given and they verify the elimination of tempering bins and conditioning and the reduction of the total energy consumed.

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HOW SUCCESSFUL IS IBP'S DUAL BED FBC? R. S. Sadowski, WEI, 225 Merrimac Street, P.O. Box 4007, Woburn, MA 01888.

This paper presents the results of the novel Wormser Grate patented dual-bed, coal-fired boiler FBC system during its initial year of operation. Its history is traced from the beginning of operation in the fall of 1982 to the present. Over this period, the steaming capacity, broiler efficiency, stack emission results and operating availability are shown, including acceptance test results and the unit's subsequent availability. Results of burning a variety of coal, ranging from low sulfur East Colorado to high sulfur (over 4% sulfur) Oklahoma bituminous, are discussed in detail. Sorbents, from Texas dolomite to Iowa limestone, are contrasted for their effects on desulfurization efficiencies. Ease of starting and normal operation load using microprocessor-based programmable controls are described and compared with gas and oil firing. Manpower requirements for operation are presented. The results have exceeded the most optimistic expectations and are certain to spark high interest among the American Oil Chemists' Society members.

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EXTENSION OF VEGETABLE OIL USING HYDROGENATED FISH OIL. L. J. Morales, I. J. Sueyoshi and R. H. y Bourges, Department of Food Science and Technology, National Institute of Nutrition "Salvador Zubirán," Mexico, D.F.

The increasing imports of seeds for the production of oils have adversely affected Mexico's balance of trade. At the same time, the national production of fish oil has greatly increased in the past 4 years because of the growth of reduction industries (fish flour). Nevertheless, the majority of this production has been destined for other industries for the production of other items, e.g., lubricants and leather curing. Countries such as Japan, Canada and Peru have been using fish oil for human consumption. Therefore, the objectives of our present work are to set up the experimental conditions to process hydrogenated fish oil and to mix hydrogenated fish oil with vegetable oil for human consumption. The experimental design consists of the characterization of crude fish oil through physical and chemical analysis; the adaptation of the procedures for refining vegetable oils for fish oil; the development of several mixtures of fish oil and vegetable oil. We obtained a hydrogenated fish oil with a melting point of 36 C, iodine index of 80, smoke point of 248 C and Lovibond color of yellow 20 and red 13. The sensory evaluation tests indicate that fish oil can be used to extend vegetable oil 30% for frying and 50% for baking without any problem in acceptability.

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ENERGY CONSERVATION IN TANKS CONTAINING VEGETABLE FATS. Alfonso Santis Monge.

A way was found of calculating heat loss in tanks without insulation. Factors that have an influence on this calculation include: the area to be covered; the temperature inside the tank; the temperature outside; the exterior diameter of the tank. Formulas were used to calculate the factors of radiation, convection and heat loss in BTU/hr. A table to determine the diameter of the insulation, using plastic clay that can be moulded, was formulated. A formula was used to determine the heat loss in an insulated tank and to determine the percentage of saved energy when insulating the tank. A way to calculate the costs of insulation, depending on the price of elastic clay was found. The time to recover the money invested in the insulator of the tank based on the subsequent energy saving was also determined.

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INSTANT NOODLES PROCESSING. S.-S. Chen, Palm Oil Research Institute of Malaysia.

The consumption and varieties of instant noodles have increased very rapidly in oriental countries in the last 10 years. The common process of noodle-making involves: mixing dough, making sheets of dough, cutting, steaming, oil frying, air cooling and packing. The oil content of the finished noodles is usually 15–20%. The process and trend will be briefly reviewed. The use and quality of palm and other oils for frying noodles will be discussed.

Session Y Oils, Proteins and By-Products from New Crops Tuesday p.m.

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EFFECTS OF PLANTING DATE AND IRRIGATION ON WAX CONTENT OF SUNFLOWER-SEED OIL. W. H. Morrison, III, USDA, ARS, R. B. Russell Ag. Research Center, P.O. Box 5677, Athens, GA 30613, Robert E. Sojka, USDA-ARS Coastal Plains Soil & Water Conservation Research Center, Box 3039, Florence, SC 29502, and Paul W. Unger, USDA-ARS Southern Conservation & Production Research Laboratory, P.O. Drawer 10, Bushland, TX 79012.

Sunflower seed grown in Bushland, Texas, in 1980 and 1981, and in Florence, South Carolina, in 1982, were evaluated for the influence of date of planting and irrigation on wax production. Wax content of the hull and oil was found to be lower in irrigated samples; however, none of the plantings appeared to be under severe moisture stress during the period from bloom to harvest. Whereas the highest correlation was found between the linoleic acid content of the oil and the wax content of the hull, those factors that favored high oil percentage also favored low wax content.

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OIL AND PROTEIN FROM OKRA (*Abelmoschus esculentus* L.) SEED. John P. Cherry, Eastern Regional Research Center, ARS, 600 E. Mermaid Lane, Philadelphia, PA 19119.

The okra plant, *Abelmoschus esculentus* L., belongs to the mallow (Malvaceae) family, as does *Gossypium* (cotton). First cultivated in Africa, okra was introduced into the US as a vegetable; the edible portion being the fresh seed pod, or gumbo. Research has resulted in the following: (a) increased amounts of oil and protein in seed to levels that are competitive with other oil seeds; (b) shatterproof dried pods with mature seeds readily harvested by mechanical means; (c) seed yields of up to 1 ton per acre; and (d) cultivars adapted to mechanical farming. Dehulled kernels from 5 varieties examined in this study contain 24.9–31.0% oil, 37.2–41.4% protein (N × 6.25), 1.8–2.5% fiber, 5.8–7.0% ash and 15.2–16.6% carbohydrate, and are good sources of vitamins B_1, B_2 and niacin,

and the minerals Ca, K, P, Fe, Zn and Cu. The oil is high in palmitic (27.4–31.5%), oleic (17.0–29.0%) and linoleic (29.5–44.4%) acids, and low in palmitoleic (0.78–0.95%), stearic (3.41–5.13%) and linolenic (0.20–0.21%) acids. The proteins have excellent essential amino acid composition, especially tryptophan, tyrosine, phenyllanine and the sulfur-containing components; the most limiting amino acid is threonine. Oil and protein products from okra seeds contribute very acceptable functional properties to food systems when substituted for similar types of ingredients from commonly used sources.

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PROPERTIES, TRADITIONAL AND POTENTIAL APPLICATIONS OF WINGED BEAN (*Psophocarpus tetragonolobus*). B. Onuma Okezie, Alabama A&M University, Office of International Programs, P.O. Box 579, Normal, AL 35762.

Not until after 1975, when the National Academy of Science published a booklet titled *The Winged Bean: A High Protein Crop for the Tropics,* did scientists and researchers begin to pay any attention to winged bean, 1 of the many underused plants of high potential economic value grown mainly in the tropical, less developed countries of the world. Today, it is the subject of considerable scientific interest in many parts of the world. Our work, and that of a few others, provides information that elucidates the distinctive characteristics of the plant as a food crop. Its leaves, flowers, immature pods, seeds and tuberous roots are used for food. Its potential food value lies in the high nutrient content of the leaves, the immature pods, the ripe seeds and the tubers. The leaves, which can be eaten in soups, stews, or cooked like spinach, are high in protein ranging from 28–35% on dmb, but low in fat content. The immature pods, with ca. 30% protein, are highly palatable and can be steamed and eaten like string beans and in salads. Compared with other oilseeds, winged bean has the highest protein content, ranging from 36–51% dmb. Its oil content ranges from 18–25%, with oleic and linoleic acids accounting for ca. 63.5%. The protein content of the tubers ranges from 10–12%, compared with 2% in potatoes and 1.5% in cassava. Our work shows that winged-bean flour can be substituted for 15–20% wheat flour with a significantly better nutritional quality than 100% wheat or 100% triticale bread without a significant change in the sensory quality of the baked product.

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COMPOSITION AND FUNCTIONALITY OF POTENTIAL FOOD INGREDIENTS. Joseph C. Scheerens and James W. Berry, Nutrition and Food Science, University of Arizona, Tucson, AZ 85721.

Buffalo gourd (*Cucurbita foetidissima*) has been investigated as a potential arid land crop produced with minimal irrigation. This species has been examined for inherent variability, response to selection and breeding, cultural requirements and for raw and processed plant product (oil, protein and starch) characteristics and their suitability for use in foods. The fruit produce seed rich in protein (30–38%) and oil (32–40%). Crude oil exhibits characteristics that are similar to oils of the linoleic acid group. Fatty acid distributions compiled from several studies reveal this material to be high in polyunsaturates (ca. 60% linoleic acid) but to possess negligible levels of linolenate. Conjugated dienoic fatty acids are present at levels approaching 2.3%. Laboratory processing yields an oil that appears to be of edible quality. The processed oil is stable and contains minimal levels of carotenoids, free fatty acids and conjugated components. Chromatographic separation and isolation of phosphatides reveal a compositional profile resembling other crude lecithins. The protein content in defatted decorticated seed meal (flour) ranges from 55–75%. Isolate preparation with base produces a protein material in yields greater than 32% of flour. Both flour and isolate proteins are most limiting in tryptophan. Whole seed possesses minimal levels of trypsin inhibitors, lectins or flatulent oligosaccharides but exhibits contents of phytate higher than those found in soybeans. Fleshy roots of buffalo gourd contain 15–20% starch. Isolated starch possesses characteristics intermediate between common root and tuber starches and cereal starches. Granules are small, often truncated and display a high level of internal organization. Amylose content, water binding capacity, swelling power and solubility most closely resemble those of cornstarch. Starch suspensions display high initial pasting temperatures and undergo a slight setback on cooling.

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CHEMICAL EVALUATION OF *Cucurbita foetidissima* SEED MEAL OBTAINED FROM OIL EXTRACTION BY AN AQUEOUS PROCESS. Vittorio Borghesi S. and Mario Calebotta D., Santiago de Chile University, Avenida Ecuador #3469 Santiago, Casilla 5659 Correo 2 Santiago, Chile.

The buffalo gourd (*Cucurbita foetidissima*) seeds represent a potential source of oil and protein, which is particularly interesting as the plant can grow in arid regions. The presence in the seeds of phytate and of a toxic bitter principle named cucurbitacin decrease its use as feed. Because these substances are soluble in water and insoluble in solvents traditionally used in the oil industry, the application of an aqueous process that simultaneously extracts oil and detoxified meal appears to be the proper technology. In this study, the chemical composition of the meal obtained in the most efficient conditions of the aqueous process was determined. Untreated seeds, presented the following composition: crude protein, 31.8%; lipids, 32.1%; fiber, 29.5%; ash, 3.6%; total cucurbitacin, 0.51%; phytate, 1.3%. Conditions found most effective for oil extraction were: degree of grinding, 46 mesh; solid to water ratio (w/v), 1:20; extraction temperature, 80–85 C; extraction time, 30 min; extraction pH, 4.5; speed of centrifugation, 14,500 rpm. Under these conditions, the meal presented the following chemical composition: crude protein, 44.1%; lipids, 1.6%; fiber, 49.3%; ash, 2.8%; total cucurbitacin, 0%; phytate, 0.2%. Simultaneous recovery of oil and the separation of undesirable compounds from meal suggest the possibility of aqueous processing being applied advantageously to buffalo gourd seed. Problems to resolve are those related to a protein fraction which becomes soluble during the process (7.8%) and to the residual content of oil (1.6%).

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AVOCADO OIL PRODUCTION: PROBLEMS AND POTENTIALS. Richard Huber, Avochem, Inc., 320 East Santa Maria, Santa Paula, CA 93060, and Itzhak Neeman, Technion–Israel, 8 Guthlevin, Haifa, Israel.

Increasing production of avocados worldwide has brought forth an infant by-products industry largely concerned with oil derived from the mesocarp of the fruit. In this paper, different countries of oil origin, the varieties of fruit and their wide variations in oil content and structural quality are discussed and worldwide oil production figures are estimated. Too, proper fruit selection, harvest timing, storage, ripening and handling are all factors that play a role in the outcome of the oil, and these are evaluated. In addition, several commercial techniques of oil extraction now being offered raise the specter of toxic components being introduced into the final product. The nature of these components and proper process conditions and techniques to avoid their incorporation are discussed, along with the relative merits of several extraction techniques from the viewpoints of capital cost, energy use, yield, quality and toxicity. Other processing difficulties are also considered. Included are literature reviews, comparison of various attributes of avocado oil with other oils and suggestions for further development. In summary, an association of producers is proposed to establish standards and to advance avocado by-product development.

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ALGAE AS SOURCES OF OIL. Stephen Lien and Kenneth G. Spencer, Solar Energy Research Institute, 1617 Cole Boulevard, Golden, CO 80401.

Various species of microalgae were reported to produce large quantities of oil and lipid as storage compounds in response to certain cultivating conditions. Most species of the oleaginous microalgae produce triacylglycerols as the predominant component of their storage oil. As in the case of seed oils, fatty acids of the algal triacylglycerols are carboxylic acids with a varying length of hydro-

carbon chain that may also include hydroxyl, keto, epoxy and other functional groups. Both conjugated and nonconjugated unsaturates, small ring systems and highly aromatic structures may also be present in varying quantities. Thus, algal oil and lipids are potential sources of renewable replacement of certain feedstock for the petrochemical industry. In order to expand both the informational foundation and the biological resources that will be needed to facilitate the adaptation of the oleaginous microalgae to produce fuels and chemicals from inorganic substrates (i.e., H_2O and CO_2), we have been conducting systematic studies to evaluate the oil-producing capacity and delineate the physiological regulation of storage lipid synthesis in algae. Over 31 strains of microalgae have been identified as potential lipid-accumulating organisms. Five of these oleaginous species have been found to be salt-tolerant strains. Lipid contents of a locally isolated strain of *Chlorella sp.* (strain S01) under nitrogen-limited growth can reach 50% on a dry weight basis.

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PROCESSING OF PRODUCTS AND BY-PRODUCTS OF FARM-RAISED CATFISH. Donald W. Freeman, USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124.

Catfish production, a relatively unknown enterprise 2 decades ago, has become one of the largest segments of the American freshwater aquaculture industry. Catfish are commercially cultivated, primarily in the south, on ca. 87,000 acres of diked water. The total production in 1982 of the major catfish processing plants was 99.6 million pounds (live weight), and the projected production for 1983 is expected to exceed 150 million pounds. A problem facing this rapidly expanding industry is waste, which comprises at least 40% of the volume of product that enters the processing plant, and contains 65–70% water. On a dry weight basis, the oil content of the waste varies from 40–60% seasonally, with the balance consisting primarily of protein and bone. This represents a potential source of nutrients of considerable value as feedstuffs. To date, profitable use of these wastes is not occurring, mainly because of the lack of technical information concerning value and alternate methods of use. Research has indicated that the wastes can be separated into oil and high protein by-products using modifications of conventional animal rendering and food-processing techniques. One promising method under study in our laboratory uses the endogenous proteolytic enzymes of catfish viscera to produce a liquid protein by-product and oil fraction. This overview will present results from laboratory and pilot-plant tests of conventional and alternate processing methods of upgrading catfish processing waste into feed supplements.

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ALKALOIDS IN PLANTS. Donald D. Bills, USDA, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

Alkaloids are basic, nitrogen-containing compounds that have pharmacological activity. They generally act as stimulants or depressants on the nervous system of animals. Some are extremely toxic, and a few are known to be mutagenic or tumorigenic. Most of the over 6,000 known alkaloids are produced by plants; only a few are biosynthesized by animals. The use of plant-derived alkaloids as drugs or mild stimulants predates recorded history. Small amounts of alkaloids are taken daily by most people in such common forms as solanaceous vegetables, beverages and tobacco smoke. The role of some alkaloids as natural insecticides or insect antifeedants in intact plants has been recognized. Despite advances in synthetic chemistry, the complex structure of most alkaloids still precludes their economic synthesis. For example, the alkaloids morphine and codeine continue to have medicinal importance and are derived entirely from the opium poppy. The value of alkaloids makes them worthy of consideration as either primary products or by-products of crop production. New plant varieties derived through breeding, cell-fusion or gene manipulation may be tailored to produce selected alkaloids, or even new alkaloids, for medical uses or as natural insect deterrents.

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PILOT TRIALS ON EXTRACTING OIL AND ALKALOIDS FROM LUPINE WITH ALCOHOLS. L. A. Johnson and J. T. Farnsworth, Food Protein Research and Development Center, F.M. Box 183, Texas A&M University, College Station, TX 77843-2476.

Lupine seeds (bitter type), an emerging crop in Europe and South America, contains 16–20% edible oil and 38–46% protein on a dry basis (db). However, 3.0 4.2% toxic quinolizidine alkaloids (dry-seed basis) prevent the meal from being used for food. The feasibilities of using either 91% isopropanol (IPA) or 95% ethanol (ETOH) to simultaneously extract oil and alkaloids using a pilot-scale continuous extractor were evaluated. Oils had to be recovered by evaporation because alkaloids do not phase separate on chilling as oil does. The meal, resulting from extraction with 4:1 solvent: Flake ratio of IPA for 60 min at 78 C, contained 1.3% oil, 64% protein, and 0.4% alkaloids (db). Although ETOH reduced the alkaloid content to 0.2% under similar extraction conditions, residual oil concentrations were much higher. The development of continuous extractors capable of operating at temperatures higher than boiling may be required to make simultaneous extraction of oil and alkaloids from lupine practical. The desolventized extracts could be phase separated into an alkaloid fraction (20%) and an oil fraction (80%) before alkali refining for greater efficiency. IPA-extracted oil contained 1% free fatty acids, 2% phosphatides and 2.7% unsaponifiables. Crude oil extracted with ETOH contained fewer free fatty acids and phosphatides, but more unsaponifiables. After treating with caustic, the oils had excellent color, and low-residual free fatty acids and phosphatides. Ca. 278 lbs oil, 101 lbs soapstock and alkaloids, 1395 lbs meal (57% protein) and 192 lbs hulls (2.25% oil) were recovered from 1 ton of seed by IPA extraction.

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EFFECTS OF EXTRUSION ON SOLUBILITY AND ELECTROPHORETIC BEHAVIOR OF COWPEA PROTEINS. R. D. Phillips and G. P. Burch, University of Georgia, Department of Food Science, Agricultural Experiment Station, Experiment, GA 30212.

Cowpeas (*Vigna unguiculata*) were extruded at feed moisture contents of 20%, 30%, and 40% and barrel temperatures of 150 C, 175 C, and 200 C. The solubility of extruded cowpea proteins was examined by sequential extractions with the following buffers: 0.1 M phosphate buffer (pH 7.0) (buffer I); 0.1 M phosphate buffer (pH 7.0), 1.0% SDS (buffer II); 0.1 M phosphate buffer (pH 7.0), 1.0% SDS, and 0.02 M 2-mercaptoethanol (buffer III). Extract aliquots were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with and without 2-mercaptoethanol. Thermoplastic extrusion under 9 different conditions caused a 7-fold decrease in the nitrogen solubility index of cowpeas. Extensive solubilization of proteins from extruded cowpea flour was accomplished with buffer II, and a subsequent extraction with buffer III solubilized much of the remaining protein. SDS-PAGE of the extracts revealed 4 polypeptide chains with molecular weights ranging from 32,000 to 115,000. The 32,000 mol wt proteins were the predominant species in cowpea extrudates extracted with buffer I, whereas the other 3 species were present in the other 2 extracts.

Session Z Anti-Nutritional Factors–Trypsin Inhibitors Wednesday a.m.

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THE EFFECT OF FEEDING SOYBEAN TRYPSIN INHIBITOR AND REPEATED INJECTIONS OF CHOLECYSTOKININ ON RAT PANCREAS. R. S. Temler, C. Dormond, E. Simond and B. Morel, Research Department, Nestlé Products Technical Assistance Co. Ltd., CH-1814 La Tour-de Peilz, Switzerland.

For 10 days, 7 groups of 10 rats were fed a balanced diet containing 18% casein as a source of protein. Two groups received soybean trypsin inhibitor (SBTI), 0.4% and 0.6% in the diet, respectively. Three groups were injected with 8 and 16 IU/kg body weight

(BW) of 95% pure CCk-39 and with a crude porcine intestinal extract containing 8 IU/kg BW of CCK. Two groups served as controls: 1 group received no SBTI, the other was injected with saline only. SBTI and CCK-39 produced similar increases in pancreatic weight, which were dose-dependent, and which lead to both cell hypertrophy and hyperplasia as measured by protein/DNA ratio and mg DNA/100 g BW, respectively. Injections of the crude intestinal extract produced at 20% higher increase in pancreatic weight, 45% more hypertrophy and 8% more hyperplasia than did 8 IU/kg BW CCK-39. The level of specific trypsin and chymotrypsin activities increased with the increase in pancreatic weight in all test groups, whereas the level of lipase specific activity remained constant. The specific activity of amylase increased only after injections of CCK-39. However, both SBTI and crude extract produced a slight decrease in amylase activity. These results suggest that SBTI and purified CCK-39 induced the same effect only on pancreatic proteases and that SBTI is a stimulator of several gastrointestinal hormones. The insulin content in the pancreas was slightly increased after SBTI only, whereas glucagon content was the same in all groups.

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THE EFFECTS OF LONG-TERM SOY- AND MILK-PROTEIN FEEDING ON THE PANCREAS OF *Cebus albifrons* MONKEYS. Lynne M. Ausman, School of Nutrition, Tufts University, James P. Harwood, FDA, Washington, DC, Norval W. King, Prabhat K. Sehgal and Robert J. Nicolosi, New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772, and Irvin E. Liener, Dana Donatucci and James Tarcza, Department of Biochemistry, University of Minnesota, St. Paul, MN 55108.

Twenty-seven juvenile cebus monkeys (*Cebus albifrons*) were reared from infancy on semipurified liquid and agar-cake diets containing 12% (as percent of calories) lactalbumin (n=8), 20% soy isolate (n=10), 13% casein (n=7) or 20% soy concentrate (n=2), for average periods of 172 ± 76, 170 ± 28, 159 ± 26 and 139 ± 22 weeks, respectively. The trypsin-inhibitor contents (mg/g) of the protein sources were: lactalbumin, 0.64; soy isolate, 2.70; casein, 0.60; and soy concentrate, 7.90. Two complete hematological and serum clinical chemistry analyses on each animal revealed no important differences in group means. Using standard surgical procedures, a 100–400 mg biopsy from the head and tail portions of each pancreas was removed for histopathological evaluation and determination of protein, RNA and DNA content as well as for trypsin and chymotrypsin activity. H and E stained sections from 26 of 27 monkeys showed normal pancreatic tissue with occasional acinar vacuolation in all diet groups. Biochemical analyses of the pancreatic biopsies of these animals appeared normal and no group differences were found between lactalbumin-, soy isolate- and casein-fed animals. No evidence of pancreatic hypertrophy, as measured by RNA/DNA ratios, was seen in any diet group. The remaining animal, 1 of only 2 fed soy concentrate, had diffuse interstitial fibrosis of the pancreas associated with mild to moderate atrophy of acinar tissue. The protein, RNA and trypsin content of the biopsy tissue of this animal was also low, probably as a result of the fibrosis. The significance of this change, however, is unknown and no conclusion can be made regarding the performance of the soy concentrate. In conclusion, long-term feeding (up to 172 weeks) of lactalbumin, casein, and soy isolate to nonhuman primates appears to provide for adequate health and development of the animal and to have no adverse effects on the pancreas.

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COMPARATIVE RESPONSE OF RATS, MONKEYS AND PIGS TO RAW OR HEATED SOY FLOUR OR CASEIN DIETS AND CHOLECYSTOKININ INJECTION. Barbara J. Struthers, G. D. Searle Co., 4901 Searle Parkway, Skokie, IL 60077.

Raw (RSF) and heated (HSF) soy flour and casein diets were compared in 4-week studies in rats, pigs and monkeys with respect to growth, pancreatic changes, fecal trypsin and nitrogen digestibility. Cholecystokinin (CCK-8) injection was compared with feeding RSF or casein to rats, and casein to pigs. Neither RSF nor HSF produced pancreatic enlargement in pigs or monkeys. Casein and HSF performed similarly. RSF produced 60% growth depression in rats, 84% in pigs, but none at all in monkeys. Pancreatic nucleic acid and protein levels in monkeys were changed by RSF, whereas, in rats, RNA/mg pancreas increased 40%, and in pigs, 20%. Changes caused by RSF in pancreatic trypsin, chymotrypsin, lipase and amylase were dissimilar in the 3 species, and RSF and CCK-8 produced dissimilar enzymatic and biochemical changes. In an 8-week study on pigs, RSF, HSF and casein diets produced the following results:

Diet protein	Casein	RSF	HSF
Body wt (kg)	29.90 b	7.07 a	29.77 b
Pancreas wt (% body wt)	0.16 a	0.17 a	0.18 a
RNA (mg/g dry wt)	120.1 ± 6.1	126.0 ± 8.4	127.9 ± 3.1
DNA (mg/g dry wt)	15.3 ± 1.6	36.4 ± 2.6	11.7 ± 0.9
Protein (mg/g dry wt)	716.1 ±17.9	580.7 ±25.3	753.4 ±16.5
Trypsin (units/mg)	156.6 ± 6.6	107.2 ±15.4	171.9 ± 9.4
Chymotrypsin (units/mg)	0.073 ± 0.007	0.018 ± 0.004	0.065 ± 0.006
Lipase (units/mg)	19.81 ± 1.89	4.04 ± 0.80	21.9 ± 2.15
Amylase (units/mg)	43.5 ± 4.1	4.9 ± 0.8	53.8 ± 5.0

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PROTEIN EFFICIENCY RATIOS OF DIETS CONTAINING TRYPSIN INHIBITOR FROM SOY AND POTATOES. E. C. Baker and J. J. Rackis, Northern Regional Research Center, 1815 North University St., Peoria, IL 61604, and M. R. Gumbmann, Western Regional Research Center, ARS, USDA, Albany, CA 94710.

Before undertaking a chronic feeding study in which rats would be fed diets containing trypsin inhibitor (TI) concentrates from soy and potatoes for 2 years, a preliminary 28-day feeding trial was run in which rats were fed casein-based diets containing these concentrates to provide 100, 200 and 400 mg TI per 100 g. For comparison, diets were included containing casein plus raw soy flour to provide the same levels of TI concentrations. In general, the protein efficiency ratio was inversely related to the TI content of the diet. The addition of either soy or potato TI concentrate at the lowest level (100 mg TI/100 g) reduced the protein quality of the diet by 6.5–14%. Pancreas weights increased with the amount of TI present in the diet, and generally were significantly higher than the casein control.

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BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF WINGED-BEAN TRYPSIN INHIBITOR. B. O. de Lumen, University of California, Room 119 Morgan Hall, College of Natural Resources, Berkeley, CA 94720.

The winged-bean trypsin inhibitor (WBTI) fraction, isolated from whole winged beans (*Psophocarpus tetragonolobus*) via affinity chromatography on trypsin-Sepharose 4B, was heterogeneous, as shown by disc gel electrophoresis. Eight protein bands, each exhibiting trypsin inhibitory activity (TIA), were separated with 2 of the major protein bands also exhibiting chymotrypsin inhibitory activity (CIA). When the WBTI fraction was subjected to $NaDodSO_4$ electrophoresis, regardless of whether mercaptoethanol was added, the heterogeneity of the fraction was reduced to 2 homogeneous bands with molecular weights of 20,900 and 16,600. With electrofocusing, the WBTI fraction was separated into 5 protein bands. The WBTI fraction was stable at acidic conditions but labile in pH greater than 8.0. At 60 C, the TIA of the WBTI fraction was not affected, but at 100 C, the thermal-stability curve was triphasic. A high level of the acidic and basic amino acids, proline,

serine, and lysine and a low level of methionine characterized the inhibitor fraction, typical of most trypsin inhibitors (TI). Sufficient quantities of TI were isolated by affinity chromatography for rat feeding. A 28-day feeding study was conducted to examine and compare the effects of feeding raw winged bean (RWB), autoclaved winged bean (AWB), and casein plus isolated WBTI on the protein efficiency ratio (PER), growth rate, and pancretic, liver and spleen weights of rats. The RWB diet, along with causing spleen an and liver atrophy, was toxic to rats, causing deaths after 12 days of feeding. The AWB diet was not toxic but growth was inhibited. The WBTI diet caused pancreatic and spleen hypertrophy and slight growth inhibition. These results will be discussed in relation to the work of others on WBTI and other antinutritional factors.

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PROTEINASE INHIBITORS FROM POTATOES: STRUCTURAL, FUNCTIONAL AND ANTINUTRITIONAL PROPERTIES. C. A. Ryan, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164.

Potato tubers are a rich source of a broad spectrum of well-characterized proteinase inhibitor proteins. Their cumulative concentrations comprise over 15% of the soluble proteins of Russet Burbank potatoes. At least 3 families of proteinase inhibitors are present in tubers that inhibit all 5 of the major digestive pancreatic proteinases that are involved in protein digestion. The inhibitors are therefore considered antinutrient proteins that are directed against the protein digestion systems of herbivores, including higher animals, insects, fungi and bacteria. Three of the inhibitors accumulate in potato leaf tissue in response to insect wounding. Thus in potato plants, these inhibitors are under environmental regulation in leaves and developmental regulation in the tubers. Structural genes for 3 of the inhibitors have been isolated from a λ phage potato gene library and are being characterized. These genes may be useful in genetically engineering plants to improve their natural plant protection systems.

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A PEPTIDE TEST FOR PANCREATIC FUNCTION AND ITS APPLICATION TO THE EFFECT OF TRYPSIN INHIBITORS ON THE PANCREAS OF THE RAT. Sang Sun Lee and Irvin E. Liener, Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, MN 55108.

The peptide N-benzoyl-L-tyrosine-p-aminobenzoic acid (BT-PABA) has been employed for the clinical diagnosis of pancreatic dysfunction in humans. This test depends on the fact that, when this peptide is administered orally, it is cleaved by chymotrypsin in the gut, and the released PABA is excreted into the urine. The amount of PABA recovered in the urine thus provides an indirect measure of the secretory activity of the pancreas. We have investigated the possibility that this peptide test might also be useful for evaluating the stimulatory effort of dietary trypsin inhibitors on the pancreas of the rat under a variety of experimental conditions without necessitating the sacrifice of animals. The reliability of this test was established by demonstrating significant positive correlations ($r=+0.8$, $P<0.01$) between the percent recovery of PABA in the urine and the weight of the pancreas and the levels of trypsin and chymotrypsin excreted in the feces of animals fed raw or autoclaved soy beans. This test proved to be particularly useful as a non-invasive technique for studying the time necessary to produce a maximum effect on pancreatic function induced by feeding raw soybeans and its reversibility by switching to autoclaved soybeans, the response of the pancreas to the feeding of an inhibitor-enriched potato concentrate, and the effect of the long-term feeding of raw soybeans on the secretory activity of the pancreas.

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INTERACTION OF DIETARY FAT AND SOYBEAN ISOLATE (SBI) ON AZASERINE-INDUCED PANCREATIC CARCINOGENESIS. B. D. Roebuck and Paul V. Kaplita, Dartmouth Medical School, Dartmouth, NH.

Consumption of raw soybean flour, which contains high levels of soybean trypsin inhibitor and unsaturated fat, enhances pancreatic carcinogenesis in rats that are treated with the pancreatic carcinogen azaserine. We examined the effects of feeding a purified soybean isolate (SBI) that contained soybean trypsin inhibitor, and a high level of unsaturated fat during the postinitiation phase of pancreatic carcinogenesis. Male Wistar rats were injected with azaserine (30 mg/kg, ip) 14 days after birth and were weaned to the test diets. Dietary composition (% by weight) of fat (corn oil) was either 5%, or, for the high fat groups, 20%. Dietary protein (20% in all diets) was supplied as either casein or heated or raw SBI. All diets were fed ad libitum for 4 months. The number and size of transections of azaserine-induced foci and the volume of pancreas occupied by these foci were determined by light microscopy. The high-fat diet increased the number and size of the azaserine-induced foci compared with the control (5% corn oil) group. The volume of pancreas occupied by foci doubled. Heated SBI had no effect on the foci. Raw SDI, however, significantly increased the number and size of foci; the magnitude of the increase in size was 2-fold greater than after the high-fat diet. The combination of raw SBI plus the high-fat diet elicited an increase in focal size that was greater than that produced by either component alone. These preliminary observations indicate that there are at least 2 components in raw soy flour contributing to the enhancement or promotion of pancreatic carcinogenesis.

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CHEMISTRY OF LEGUME PROTEASE INHIBITORS AND THEIR USE IN TAXONOMY. Juergen K. P. Weder, Institut fuer Lebensmittelchemie, Technische Universitaet Muenchen, Lichtenbergstr. 4, D-8046 Garching, Federal Republic of Germany.

Proteins inhibiting proteolytic enzymes, especially trypsin and chymotrypsin, are widely distributed in *Leguminosae (Fabales)*. A considerable number of these inhibitors have been isolated from legume seeds. The chemical characterization of these proteins has been carried out to varying extents. Data on the primary structure and reactive sites known for some of them allow a classification into inhibitor families; inhibitors with the same topological molecular structures are grouped together. Evaluation of amino acid sequences in the same inhibitor family in terms of amino acid replacement or mutation of bases in the corresponding deoxyribonucleic acids offers the possibility to understand taxonomic relations at a molecular level. Furthermore, some of the structures are related to one another giving information on phylogenetic relationships. With less laboratory effort, inhibitor patterns can be obtained by disc electrophoresis of seed extracts and specifically staining the inhibitor bands. The types of inhibitor patterns can be used for taxonomy in combination with trypsin and chymotrypsin inhibitor activities and the quotients of the 2 activities. Results obtained with this method were first proven to correlate with the results of other chemotaxonomic techniques in the genus *Acacia*. In the context of Australian *Acacias*, this technique was then used to demonstrate closer relationships between uninerved *Racemosae* and *Botryocephalae (Bipinnatae)* and also between *Plurinerves* and *Pulchellae (Bipinnatae)* than between the 2 *Bipinnatae* and between *Plurinerves* and *Juliflorae*. This technique has also been used to exclude *Acacia mitchellii* from the series *Pulchellae*.

Session AA Physical Properties of Oil Wednesday a.m.

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ADSORPTION OF SOY OIL PHOSPHOLIPIDS ON SILICA. Helen G. Brown and Harry E. Snyder, University of Arkansas, Food Science Department, Route 11, Fayetteville, AR 72701.

An experimental method for the adsorption of phospholipids from soybean oil was developed based on chromatographic properties of the oil components. The traditional method for removing phospholipids involves hydrating the gums. However, when crude oil in hexane is applied to thin layers or columns of colica, the phospholipids irreversibly adsorb. The triglycerides can be eluted with

nonpolar solvents and phospholipids with a polar solvent system. Hence, a basis exists for a selective adsorption of phospholipids on silica. The system involved stirring 100 ml of oil in solvent (i.e., miscella) with 1 gram of silica for 15 min. The phosphorus content, before and after the reaction, was analyzed by wet ashing and Fiske-Subbarow colorimetric reaction. Addition of isopropanol (at least 1%) to the hexane miscella caused an increase in phosphorus adsorption, most likely caused by liberating triglyceride from adsorption sites. Increased adsorption was achieved by deactivating the silica. Oil concentration did not appear to affect the adsorption. The amount of phosphorus adsorbed was dependent upon concentration of the phospholipid. When phospholipid adsorbed per gram of silica is plotted vs the residual phospholipid, the plot resembles a Freundlich isotherm for reversible adsorption. Yet the adsorption is irreversible. Possible explanations for this type of adsorptive behavior will be explored.

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COMPUTER MODELING OF α- TO β'-FORM PHASE TRANSITIONS USING THEORETICAL TRIGLYCERIDE STRUCTURES. James W. Hagemann, Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture, 1815 N. University Street, Peoria, IL 61604.

Selected bond rotations on theoretical triarachidin α-form structures produce bent β'-forms that have long spacings that were previously reported. Intermolecular minimization procedures determined the positions that best fit the β'-forms around a centralized molecule in 9 different subcell arrangements. Computer-drawn graphics of the molecular packing revealed that 5 of the subcell arrangements had unfavorable asymmetric molecular spacing. Although interactions across the methyl gaps amount to only 2–3% of the total energy in final α- and β'-forms, computer-generated energy profiles during α- to β'-phase transitions revealed high repulsive energy regions from the close approach of methyl groups. This observation, plus additional repulsive interactions between the side packing of molecules during rigid chain rotations, necessitated the modification of certain chain movements during the phase transition to reduce excessive repulsive energy. These results suggest that phase transitions proceed in a particular sequence of events that most efficiently distribute the energy to promote further phase excitation or that lead to collapse into a stable polymorphic form. The total phase transition energy curves also reveal that other β'-forms, in addition to those originally found from chain rotations of α-forms, are possible and are dependent on the starting α-forms, the direction of chain rotation and the subcell arrangement.

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THERMAL ANALYSIS AND ITS APPLICATION TO LIPID PHASE TRANSITIONS. James W. Hampson, Eastern Regional Research Center, ARS, USDA, 600 E. Mermaid Lane, Philadelphia, PA 19118.

The phase transitions and thermal behavior of pure and mixed lipids studied by differential scanning calorimetry will be discussed. The phase diagram of methyl oleate/methyl palmitate, showing eutectic formation as well as the effect of polymorphism, will be illustrated. Binary and ternary diagrams of POS, POP and SOS will show the importance of tempering in obtaining the proper crystal form. The binary phase diagram of the optically active (cryptoactive) PPO triglycerides will be presented with its unusual behavior. Specific heat in relation to lipid phase will also be shown.

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STRUCTURAL MORPHOLOGY OF BLOOM ON CONFECTIONERY COATINGS PREPARED WITH COCOA-BUTTER EXTENDERS. Robert Delaney, Durkee Foods Division/SCM Corporation, 16651 Sprague Road, Strongsville, OH 44136.

Microstructural investigations of fat crystals may lead to a better understanding of the thermodynamically stable forms of confectionery fats. X-ray diffraction and scanning electron microscopy studies of commercially available cocoa-butter extenders (CBEs) in typical confectionery coatings exhibit similar physical and chemical behavior to that of cocoa butter (CB). Samples of severely degraded coatings were evaluated to illustrate the structural similarities of the coatings. The effect of processing and compatability of different fats are observable by the occurrence of solid-state disruption in the formation of nucleated, fat-rich sites (bloom). This disruption of triglycerides is substantiated by partial fractionation and recrystallization to stable polymorphic forms on the surface and throughout the coating mass. Unlike cocoa butter, cocoa-butter extenders generally exhibit fewer polymorphic forms so that the most stable form occurs with normal tempering. Blood formation of these systems may, therefore, occur by a mechanism other than polymorphic transformation.

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ELECTRON MICROSCOPY AND SUPPORTIVE TECHNIQUES USEFUL IN THE STUDY OF FORMULATED FOOD SYSTEMS. E. A. Davis and J. Gordon, Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108.

The application of scanning and freeze-fracture electron microscopy and differential scanning calorimetry (DSC) techniques toward a better understanding of the physicochemical properties of formulated food systems containing oil and emulsifiers will be illustrated using batter systems. Micrographs showing how oil and emulsifiers disperse and interact with the components of a batter system before, during and after heating will be discussed. Temperature of phase transitions and enthalpy values calculated from DSC curves will be presented for selected oil, emulsifier and starch batter systems.

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COMMERCIAL MARGARINE PRODUCTS—AN ANALYTICAL SURVEY. Y. K. Teah, M. S. A. Kheiri, Karimah Admad and K. G. Berger, Palm Oil Research Institute of Malaysia, P.O. Box 10620, Kuala Lumpur, Malaysia.

A large number of samples of margarines from East and West Europe, Asia, Australia and North America have been examined for their physical behavior and their physicochemical characteristics. Their proximate composition has been deduced. Certain general conclusions could be drawn. The physical behavior of the products conformed to the ranges of consistency defined by Haighton, JAOCS (1969) 36, 345, in most cases, provided the normal local temperature of use was taken into account. However, in some instances the incorporation of locally available ingredients at high levels resulted in excessively firm textures. The functionality of palm-oil products as margarine ingredients was investigated.

Session BB Automation and Process Control Wednesday a.m.

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AUTOMATED SOLID FAT CONTENT MEASUREMENT USING A MICROCOMPUTER-CONTROLLED ROBOTIC SYSTEM. Bryan L. Madison, The Procter & Gamble Company, Winton Hill Technical Center, 6071 Center Hill Road, Cincinnati, OH 45224.

This work will describe the development of a robotic system to automate the measurement of samples for solid fat content using a Praxis SFC 900 pulsed NMR instrument. A Microbot Alpha robot was mounted on the instrument and does all the required sample tube transfers. An Apple II microcomputer provides the required intelligence for controlling the robot, defining tempering conditions, data acquisition, calculations and data storage. The impact of the robotic device in the areas of data precision and accuracy, overall reliability and cost/time savings will be assessed. A short videotape of the application will be shown.

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OIL STABILITY INDEX. Allan Buck and Mark Matlock, Archer Daniels Midland Co., 1251 Beaver Channel Parkway, Clinton, IA 52732.

A precise, fully automated method for determining the stability

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of vegetable oils is described. This method was designed solely for the purpose of improved quality control. The method is based upon the modified Swift Test developed by Pardun and Kroll in which the stability of an oil is determined by measuring the increasing conductance produced during accelerated oxidation when volatile acids are collected in deionized water. Current methodology had severe limitations regarding sample processing and subjective endpoint determination. We took the analytical technique and expanded it to 16 samples using disposable glassware. The use of a microcomputer allows all samples to be started, stopped, and run independently. The results are continually stored in memory and may be displayed at any time during the run. A pen plotter allows overlay of plots for comparisons of samples run at different times on the instrument. Furthermore, we incorporated a software technique with which the computer determines the autooxidation time, rather than have the analyst make a subjective determination by drawing trangents and so forth. The instrument has built in diagnostics that allow it to identify any malfunctions. We have used this instrument to follow the oil stability throughout the refining process, and we will present these findings here. We refer to this test as the Oil Stability Index or OSI.

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APPLICATION OF A COMPUTER-BASED DATA ACQUISITION SYSTEM IN A SOAP MANUFACTURING PILOT PLANT. Charles F. Irwin and Thomas B. Dignan, Lever Brothers Company, 45 River Road, Edgewater, NJ 07020.

The Development Department of the Lever Brothers Company Household Products Division has installed a computer-based data acquisition system in its soap manufacturing pilot plant. The system is vertically and functionally distributed and is comprised of 4 remote 8-bit Z80 microprocessors and 2 centralized host 16-bit INTEL 8086 microprocessors with dual floppy disks. The system simultaneously supports up to 4 independent areas with their operator work stations and acquires data from 400+ sensors at a maximum rate of 200 channels per second. It costs only $200 per channel, provides over 700 kb of processor power, and was operating in 4 months. The system allows the operators to concentrate on qualitative aspects of the process rather than in collecting data and makes possible the study of system transients and process parameters that were previously neglected. These functionalities are invaluable in conducting batch process development experiments. The Z80 micros are located locally in each of the 4 areas to improve signal accuracy and reduce wiring costs. They continuously scan the sensors, converting the analog signals to a digital representation, and transfer the data to their 16-bit host. The host performs engineering unit conversions, limit checks and alarms, supports the remote CRT and printer operator interfaces and stores the data on disk. The data files for each run are then transferred to a VAX 11/750 for statistical analysis and report generation. The system is driven by tables prepared on the VAX and downloaded to the microprocessors. It may be reconfigured or expanded simply by editing the control information table; the software need not be changed. Hence, the system is flexible and easily used in other data acquisition applications. The system is also very user friendly because it is menu driven. Operators control all aspects of the system operation simply by selecting items from a menu.

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AUTOMATION IN AMERICA'S REFINERIES. Stanley C. Loft, Johnson-Loft Engineers, Inc., 3100 Kerner Boulevard, Suite C, San Rafael, CA 94901.

With the introduction of microprocessor chip technology, the edible oil industry has come to realize the potential of microprocessor control as a means of reducing operation costs, increasing efficiency and improving final product quality. This paper will present automation concepts practiced today in modern refineries, and will review the limitless potential for process control using today's affordable microprocessors and mini programmable controllers.

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THE APPLICATION OF VERY HIGH ACCURACY GC ANALYSIS AS AN AID TO FAST AND RELIABLE CONTROL OF OIL REFINERY OPERATION. J. D. Craske, C. D. Bannon, Ngo Trong Hai, J. Denmead and K. O'Rourke, Unilever Australia Limited, Box 9, P.O., Balmain, NSW 2041, Australia.

If we consider the analytical techniques that were available to lipid chemists as little as 25 years ago, we may characterize them generally as slow, relatively inaccurate and giving little information because they so often measured only the average properties of complex mixtures. With the development of chromatographic techniques, particularly gas chromatography and, more recently, high performance liquid chromatography, the information delivery of analysis systems has improved dramatically, even to the point of determining individual components in highly complex lipid mixtures. This power has revolutionized our ability to advance our knowledge of lipids in research laboratories. However, the full power of research techniques are seldom needed for factory control. In this area, the accent is on speed, accuracy and reliability and the delivery of information that is highly relevant to the control of unit processes and product quality. Here, the computer is playing a crucial role. The lecture will summarize a program that has been carried out greatly to improve the accuracy, reliability and speed of gas chromatographic analysis of fatty acid methyl esters and, together with the computer, to speed up and improve interpretation of the results as a means of providing close to online control of edible oil refinery operation. Total time for analysis and interpretation of results is ca. 15 minutes.

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PROCESS CONTROL AND AUTOMATION OF EDIBLE OIL REFINERIES. D. W. Foster and A. J. Harper, Simon-Rosedowns Limited, Cannon Street, Hull HU5 0AD, United Kingdom.

The paper begins with references to the objectives of process control and automation in refinery operations. Process control has progressed from earlier pneumatic systems with electronic relay logic to the use of electronic analog control with microprocessor-based logic systems. Many of the control instruments now used are microprocessor based. Automation has further progressed to the concept of computer control of total refinery operations. The merits of direct digital control with a central microprocessor vs distributed systems are discussed. The paper indicates the relative costs of the different types of control system and postulates future trends in edible-oil refinery control. The progress in refinery automation is illustrated by reference to its application to bleaching, deodorizing and other refining operations.

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AUTOMATION IN THE OIL MILLING INDUSTRY. Kari Jokinen, Raision Tehtaat, Finland.

The paper is based on the experiences gained in Raision Tehtaat when automating the oil mill. First, I briefly describe automation in general. The fundamentals of the use of automation, the connection between process planning and automation planning, the selection of equipment and requirements on the equipment will be handled next. Finally, I will look into the posisbility of using production control systems for serving business and production management.

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WHAT OPERATIONS MANAGEMENT SHOULD KNOW ABOUT PROCESS CONTROL SYSTEMS. Bob Babecki, Foxboro Canada Inc., 707 Dollard Ave., La Salle, Quebec H8N 1S5, Canada.

Operations management has the responsibility to evaluate the risks and benefits of implementing process control systems. This paper outlines some of the more important points that management should cosider in an evaluation, regardless of the technology used to perform the system functions. Based on a wide range of application experience, important benefits resulting from the use of control systems are discussed to aid economic justification. Factors that tend to increase risk and areas of special concern are examined to help plan successful implementation.

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Session CC Novel Developments in GLC Wednesday a.m.

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NEW DEVELOPMENTS IN GAS LIQUID CHROMATOGRAPHY. Wolfgang Bertsch, Chemistry Department, University of Alabama, P.O. Box H, University, AL 35486, and A. A. Spark, Laboratory Analysis Services, 99 Hope Street, Cape Town, South Africa.

Capillary (or open tubular) gas chromatography (GC) has gained further importance over the last few years. The advances made since 1978 are more significant than those in the previous 20 years. With the exception of applications based on gas-solid interactions and of preparative scale separations, GC in capillary columns can be performed faster and at lower detection limits than in packed columns. Improvements in GLC hardware primarily fall into the following categories: (a) Column technology. The most significant advances have been made in the preparation of thermolabile capillary columns of outstanding inertness. The combination of flexible fused silica with immobilized phases of variable film thickness has now made possible separation that could not be accomplished before 1978. (b) Sample inlets. Rediscovery of cold on-column injection has changed methodology for analysis of low-volatility samples and of samples having a wide boiling point range. Long considered as the weakest part of capillary GC, these cold sampling methods do not only extend the range of GC but also greatly improve analytical precision. (c) Special instrumental adapters. Several devices for the microozonization and microhydrogenation have been introduced and applied to the analysis of glycerides. Analytical methodology, e.g., new approaches to automation, optimization and sample preparation, has also progressed. The recent developments of interest to the oil chemist will be discussed and illustrated with selected application examples.

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COMPOSITIONAL ANALYSIS OF COMMERCIAL FATS AND OILS BY CAPILLARY GAS CHROMATOGRAPHY AND TRADITIONAL METHODS. Bryan L. Madison, The Procter & Gamble Company, Winton Hill Technical Center, 6071 Center Hill Road, Cincinnati, OH 45224.

This work will describe the application of capillary gas chromatography (GC) and traditional procedures for the compositional analysis of commercial fats and oils. The *trans* isomer content, iodine value and *cis,cis*-methylene interrupted polyunsaturated fatty acid (PUFA) content of a fat or oil can be calculated from a GC analysis of fatty acid methyl esters (FAME) on a Silar 10C capillary column. An automated derivatization procedure that uses the Zymate laboratory robot will be discussed. The areas of data precision and accuracy, overall reliability and cost/time savings will be assessed.

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RECENT ADVANCES IN FUSED SILICA COLUMN TECHNOLOGY. R. R. Freeman, Hewlett-Packard Company, Route 41, Avondale, PA 19311.

The first fused silica open tubular columns were introduced in 1979. These columns are characterized by their inertness and flexibility. The ease with which these columns are installed have encouraged many to incorporate these columns into routine use. The inertness of the columns has broadened the range of compounds that can be analyzed via high resolution gas chromatography. Over the past 4 years, 3 major advances have contributed to the widespread use of these columns. The glass drawing technology has improved. Present day columns are mechanically more durable than their predecessors. Columns are available with internal diameters of from 0.2 mm to 0.53 mm. The small diameter columns are noted for their high efficiency (ca. 5,000 plates/meter). The large diameter columns have sample capacities on the order of 2–5 μg/component. Many stationary phases are now cross-linked. The cross-linked phases yield a column that is extremely durable—often in service for years. When contaminated, the column can be rinsed with practically any solvent. The third major advance is the column-to-column reproducibility possible with a fused silica column. The manufacturing processes have been refined so that column performance can be very precisely determined and maintained. This stability has made qualitative analyses using high-performance fused silica columns a reality. Each of these advances will be discussed. Chromatograms of volatile fatty acids, fatty acid methyl esters and triglycerides will be used to illustrate specific points concerning column performance, stability, and reproducibility.

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THE POTENTIAL OF CARBOWAX-20M BONDED IN FLEXIBLE FUSED SILICA WCOT COLUMNS AS THE STANDARD FOR INTERLABORATORY COMPARISONS OF FATTY ACIDS. R. G. Ackman, Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax, N.S. B3J 2X4, Canada.

In GLC analysis of natural all-*cis* fatty acids, the polarity of packed columns depends on the type of liquid phase, its proportion on the support, and the temperature. An infinite range of polarities has been reported, but because of chain length overlap, most widely used polar-packed columns create identification problems among the C_{18}, C_{20} and C_{22} polyunsaturated fatty acids of special interest to biomedical researchers. Interlaboratory comparisons of fatty acid compositions are often confusing or incomplete. With open-tubular columns, the greater efficiencies partially offset this problem of identifications or peak coincidence, as shown by our past publications on the liquid phase BDS polyester and on moderately polar cyanosilicones. Despite the simplicity of such columns, a few problems of polarity remained. In the last decade, open-tubular columns with Carbowax-20M, coated first in glass and later in flexible fused silica, have pointed the way to a column of moderate polarity, giving excellent interlaboratory reproducibility of retention data. With the introduction of Carbowax bonded to flexible fused silica, columns with highly desirable stability and operating flexibility have been achieved. Readily available standards, such as concentrates of fish oil and canola oil, allow any laboratory to quickly confirm retention data tabulated for polyunsaturated fatty acids of the types found in all animal organs. $AgNO_3$-TLC provides further confirmation if attention is paid to isomer subfraction effects.

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ANALYSIS OF AUTOXIDIZED FATS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY: HOMOLYTIC CLEAVAGE PRODUCTS OF MONO- AND DIHYDROPEROXIDES AND BICYCLOENDOPEROXIDES OF METHYL LINELENATE. E. N. Frankel, W. E. Neff and E. Selke, Northern Regional Research Center, 1815 N. University Street, Peoria, IL.

To elucidate the genesis of volatile lipid oxidation products, thermal homolytic and acid heterolytic decomposition processes were compared. Primary and secondary oxidation products were decomposed thermally (200 C), and the volatiles formed were identified by capillary gas chromatography-mass spectrometry (GS-MS). Oxidation products were also decomposed in the presence of HCl-methanol, and the resulting dimethyl acetals were identified by GC-MS. The volatile thermal decomposition products were those expected by homolytic β-scission on both sides of the hydroperoxide group. No dialdehydes were identified under our thermal decomposition conditions. In contrast, the acetals formed under acid decomposition conditions were those expected by heterolytic scission only between the hydroperoxide group and the allylic double bond. Dialdehydes identified from acid decomposition of cyclic peroxides and dihydroperoxides included malonaldehyde and 3-hexenedial. The biological and flavor significance of these volatile lipid oxidation products are under further investigation.

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SEPARATION AND DETERMINATION OF TRIGLYCERIDES ACCORDING TO CHAIN LENGTH AND DEGREE OF UNSATURATION BY COMBINED LIQUID CHROMATOGRAPHY/

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GAS CHROMATOGRAPHY. Del E. Moore, Durkee Foods, SCM Corporation, 16651 Sprague Road, Strongsville, OH 44136.

A method is described that characterizes all triglyceride components of nonlauric vegetable fats by carbon number (CN) and total double bonds using liquid chromatography (LC) for separation and collection and gas chromatography (GC) to quantitate the collected LC fractions. The advantage of this method, compared with other hyphenated techniques, is a high degree of characterization (perhaps 30 components) in a relatively short time. When a GC autosampler and suitable computer program for data handling and hard copy output is available, sample throughout of 1/2 man per day per sample can be achieved. This paper will describe an LC separation made on a C-18 heated column using a CH_2Cl_2/acetonitrile mobile phase. A Foxboro/DuPont IR detector is enclosed in a sealed, dry air-purged plexiglas cabinet. The detector output, through special signal processing, permits operation at 0.01–0.02 AUFS @ 5.7u with less than 1% noise level. A special regeneration cycle has extended LC column life more than 1.5 years. A gradient-forming pump is not needed in this analysis. Depending on sample type, baseline resolution of sample components containing ECN (ECN (Equivalent Carbon Number) = CN – 2 (double bonds)) 44–54 is routinely obtained.

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SEPARATION OF *cis/trans* AND POSITIONAL ISOMERS OF UNSATURATED FATTY ACID METHYL ESTERS BY CAPILLARY GAS CHROMATOGRAPHY. E. Schulte, Institut fur Lebensmittelchemie, University of Muenster, Piusallee 7, D-4400 Muenster, West Germany.

Hydrogenation of nutritional fats can be detected by determining *trans* fatty acids. The separation of the *cis*- and *trans*-octadecenoic acids was obtained with short capillary columns coated with apolar stationary phases. With long capillaries, containing the same phases, separations of positional *trans*-isomers were also possible. Polar columns with cyanopropylsilicones give a better separation of the positional isomers, but a partial overlap of the *cis*- and *trans*-isomers occurs. Therefore, the combination of an apolar and a polar column connected by a column switching system was used. In this way, the *cis*- and *trans*-fractions were transferred from the first to the second column separately, where positional isomers could be resolved. This method was applied to hydrogenated and autoxidized plant oils and to butter fat. The relationships in concentrations of the different isomers were determined. The method described here is not very sophisticated and can be used for routine analysis.

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SPECIAL TESTING IMPROVES EQUIVALENT CHAIN LENGTH REPRODUCIBILITY FOR FAME CAPILLARY COLUMNS. Leonard M. Sidisky, Paul H. Silvis and William F. Fatula, Supelco, Inc., Supelco Park, Bellefonte, PA 16823.

Analysts have recognized the importance of using equivalent chain length (ECL) values to predict the identity of fatty acids in natural samples. A major problem when using ECL values has been column-to-column variability, making it difficult to rely on ECL identifications–particularly when interlaboratory comparisons are being made. Only by minimizing column-to-column variability can ECL data become more reliable. A test mixture was devised to screen capillary columns and allow the determination of the ECL values. The test mixture also allows other column quality parameters, e.g., efficiency and inertness, to be determined. By using the test mix to screen capillary columns, stringent specifications can be placed on capillary columns that will improve column-to-column reproducibility and give the analyst more confidence when using ECL values. Supelcowax 10 (boned CARBOWAX) was used with this test mixture because it elutes FAME according to carbon number and number of double bonds. Chain overlap, which often occurs with cyanosilicones such as Silar 5 CP or SP-2300, is minimized with the specially tested Supelcowax 10 capillary column. Complex FAME samples that contain a variety of saturated and unsaturated components will be shown, analyzed on a Supelcowax 10 column and a variety of cyanosilicone capillary columns to compare predicted ECL values with the actual ECL values. High thermal stability was another reason Supelcowax 10 was chosen for special testing. Data will be presented that shows the increased thermal stability of Supelcowax 10 over both conventional nonbonded CARBOWAX 20M and the cyanosilicone phases. The higher thermal stability of Supelcowax 10 allows the analyst to analyze FAME at higher temperatures than with nonbonded CARBOWAX 20M, thus reducing analysis time. Other advantages of bonded phases, such as the ability to be solvent rinsed, will also be discussed.

Session DD Surfactant and Detergents: Symposium on Wetting Wednesday a.m.

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EFFECT OF MOLECULAR STRUCTURE ON THE ABSORPTION OF ALKYLARYLSULFONATES AT AIR-WATER OR HYDROCARBON-WATER BOUNDARIES. John J. Meister, Southern Methodist University, Department of Chemistry, Dallas, TX 75275.

Studies on the boundary (surface and interfacial) tension of aqueous solutions of hydrocarbon sulfonates show that the structure of the sulfonate molecule has a pronounced effect on the capacity of the sulfonate to reduce boundary tension. By considering a large body of boundary tension data in the literature, one can predict that certain molecular structures are more effective than others in populating a solution interface and, thereby, reducing boundary tension. Within specific molecular weight limits, aqueous solutions of di(branched alkyl)-arylsulfonates and di(n-alkyl)arylsulfonates, both structures with the hydrophilic group in the center of the molecule, will provide maximum excess absorption at an aqueous solution boundary. This produces a minimum boundary tension between the sulfonate solution and air or hydrocarbon. The structural characteristics that make these molecules exhibit maximum interfacial activity are: (a) that the sulfonate group be attached to the aromatic ring next to a long alkyl chain, (b) that the alkyl portion of the molecule consist of at least 2 parts of equal length which are placed at equally spaced locations around the aromatic ring and (c) that the alkyl chain be branched.

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WETTING KINETICS: AN OVERVIEW. P. Neogi.

Abstract not available.

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PHYSICO-CHEMICAL ANALYSIS OF THE RESERVOIR WETTING PROBLEM. J. C. Melrose, Mobile Research and Development Corp., P.O. Box 819047, Dallas, TX 75381.

The stability of thin aqueous wetting films is believed to be an important factor in determining wettability conditions in petroleum reservoirs. An initial attempt to apply overlapping double layer and dispersion force theory to this problem has recently been reported (SPE 10971). This analysis required an evaluation of the net force vs thickness relationship for electrolyte concentrations in the range of 0.05–0.3 N. In the present paper, both the attractive and repulsive components of the net force are treated in more detail. The various approximations and assumptions used in this treatment are examined, and relevant data from the scientific literature are reviewed. Film thickness and film stability are shown to be related to pore size. That a critical value of the wetting-phase saturation can be defined is also shown. This critical saturation is the value of the saturation at which stable wetting films are no longer maintained on the pore surfaces of the desaturated pore volume. Geological factors that tend to produce saturations less than the critical value are discussed.

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WETTABILITY MODIFICATION OF METAL OXIDES BY ALCOHOL ADSORPTION. William H. Wade, University of Texas at Austin, Welch 3.418, Austin, TX 78712.

The gas phase adsorption of low molecular weight alcohols

(C_1–C_4 OH) on well outgassed metal oxide surfaces is quite strong. Molecular areas are consistent with normal orientation on the surface and the hydrocarbon tail lengths used are sufficient to isolate the underlying substrate and replace it by one that is paraffinic in character. The resulting decrease in wettability is evident in the alcohol absorption isotherm as well as in the desorption branches of hysteresis loops if mesoporous substrates are used. Finally, it is also evident in the spontaneous imbilition of these alcohols in Porous Vycor®.

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WETTING OF POWDERS AND ITS RELEVANCE TO THE DISPERSION PROCESS. Geoffrey D. Parfitt and Raul Ayala, Carnegie-Mellon University, Department of Chemical Engineering, Pittsburgh, PA 15213.

Several aspects of the process of dispersing colloidal powders in liquids that are related to systems used in technology lack fundamental understanding. For convenience, we may divide the overall process into 2 stages. The first is concerned with incorporation, wetting and disagglomeration leading to a uniform distribution of fine particles throughout the medium. The second stage involves flocculation, which is normally controlled by charge and steric effects (caused by adsorbed polymer). Effective wetting is an important prerequisite for efficient disperson. This involves both the external and internal surfaces of the powder agglomerates, and is dependent on the nature of the liquid phase, the character of the solid surface, the dimensions of the interstices and the nature of the process used to bring together the components of the system. Several techniques have been developed to study powder wetting, and used with varying degrees of success. This paper will consider the forces involved in wetting and how they relate to the chemical and mechanical energy required to break down agglomerates into primary particle disperson.

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OBSERVATIONS ON WETTING CONDITIONS INDUCED BY CRUDE OIL. Jill Ward and Norman R. Morrow, New Mexico Petroleum Recovery Research Center, New Mexico Institute of Mining and Technology, Socorro, NM 87801.

Reliabile control of oil-water wetting behavior at high energy surfaces is essential for laboratory investigations into the effect of wetting on oil recovery from sedimentary rocks. To this end, the wetting alteration properties of crude oils have been studied. If crude oil is used as the oleic phase, characterization of wettability by contact angle measurement at plane surfaces is often complicated by formation of rigid films limits on measurable contact angle hysteresis caused by drop instability and inconsistencies in the induction times required for oil adhesion at surfaces initially covered by water. Wettability alteration is commonly ascribed to films formed by irreversibility adsorption of components from the asphaltene and resin fractions of the oil. Studies have therefore been made of the wetting characteristics of silica surfaces treated by exposure to selected crude oils. Advancing and receding contact angles exhibiting considerable hysteresis have been measured on the treated silica substrates for a variety of pure hydrocarbons against water. Factors considered included the exposure time, subsequent cleaning and storage procedures and use of deasphalted crude oil. Film deposition, applied to 2-dimensional glass capillary networks (micromodels) and to sandstones, was used to investigate the effects of wettability on fluid distribution and displacement behavior.

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SOIL WETTING AGENTS FROM OXYETHYLATED AMIDES. Thomas J. Micich and Warner M. Linfield, USDA-ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

Preliminary work to prepare and evaluate moisture control agents for soil was done with polydisperse adducts derived from oxyethylated secondary amides, $RCON(R')(CH_2CH_2O)_nH$, where R and R′ are aliphatic or aromatic groups containing 12–20 carbon atoms and n has values from 5 to 20. Attractive wetting properties were observed with cotton skeins and generally confirmed with pressed peat moss. Aliphatic R groups yield better wetting agents than aromatic groups with the most effective wetting properties occurring with short ethylene oxide chain lengths. Unreacted amide was found in most of the adducts but was present to a lesser degree in adducts from aromatic amides. Oxyethylation of N-hydroxyethyl secondary amides eliminated the problem of low amide reactivity. The absence of free amide resulted in marked improvements in wetting properties. Optimum wetting ability, maximum surface activity and complete water solubility generally occurred with adducts containing less than 10 mol ethylene oxide.

Session EE Analytical Applications of Supercritical Fluids Wednesday a.m.

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SUPERCRITICAL FLUID CHROMATOGRAPHY MEASUREMENTS–IMPLICATIONS FOR CRITICAL FLUID EXTRACTION. J. W. King, CPC International, Moffett Technical Center, Argo, IL 60501.

The last decade has seen the further development of supercritical fluid chromatography (SFC) as a viable analytical tool for the characterization of complex mixtures and for the determination of physicochemical data. Unfortunately, the complementary role of SFC to critical fluid extraction (CFE) has not always been appreciated and there exists some confusion as to how this technique supplements its extraction analog. This presentation critically examines the role that SFC plays in providing additional information relevant to CFE. An examination of the available literature suggests that SFC can be applied over the same range of reduced state parameters as CFE. Hence, valuable information can be extracted from the pressure dependence of chromatographic retention parameters and the variance in plate height with gas density. Examples of the measurement of solute solubility in the gas phase and the determination of diffusion coefficients via chromatographic techniques will be cited. Analogies will be drawn between the use of modifiers in SFC and the incorporation of entraining agents in the field of CFE. A speculative use of SFC for the determination of phase boundaries will also be proposed. Differences between the SFC experiment and the conditions under which CFE is normally conducted will be discussed. The presence of the stationary phase in many chromatographic studies tends to complicate the interpretation of SFC results because such effects as competitive adsorption at the gas-solid interface and the salting out effect in gas-liquid systems. As a consequence, there is a crucial difference between solubility enhancement and chromatographic migration enhancement. The high sensitivity of chromatographic-based detectors can also lead the experimentor to erroneous conclusions regarding the finite solubility of a specific solute in the critical fluid and in addition introduces a technique dependence into the concept of threshold pressure as defined in SFC and CFE. Despite the above precautions, quasi chromatographic techniques, e.g., frontal analysis and perturbation chromatography, offer a rapid method of obtaining basic solubility data for compounds undergoing dissolution in critical fluids. Typical equipment required to use the above techniques will be discussed and examples of SCF/solute systems studied via the above methods presented.

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DENSE GAS SOLUBILITY MEASUREMENTS. L. M. Bowman, Jr., Syntex Ophthalmics, 2328 West Royal Palm, Phoenix, AZ 85021, J. C. Giddings and M. N. Myers, University of Utah, Department of Chemistry, Salt Lake City, UT 84112.

The solubility of several compounds in a molecular weight range from 200–4,000 have been measured in dense CO_2, ethane, fluoroform, 1,1-difluoroethane, and SF_6. Dense gas solubility has been shown to be a parabolic function of the gas density by applying Hildebrand's solubility parameter concept. Data on the absolute solubility, threshold pressure, effective volume, and solubility

parameter for each compound studied will be discussed. A comparison between dense gas and liquid solubility, as well as solubility as a function of temperature, will be presented.

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SUPERCRITICAL FLUID CHROMATOGRAPHY. D. R. Gere, Hewlett-Packard, Avondale Division, Route 41, Avondale, PA 19311.

The use of supercritical fluid chromatography (SFC) has undergone a significant increase in the past few years. Several reasons can be found for this renewed interest in a technique that is ca. 20 years old. This paper will discuss the application and use of small diameter packed columns using carbon dioxide as a mobile phase. SFC an an analytical technique is positioned as a complementary separation method to gas chromatography (GC) and high performance liquid chromatography (HPLC). Thus, SFC is relevant to separation problems that overlap the other techniques. A supercritical fluid mobile phase takes advantage of favorable diffusivity, viscosity and dynamically variable solvent power (via density adjustment) to achieve significant differences in resolution and resolution per unit time. Examples will be shown to illustrate the higher degree of resolution per unit time than certain specific HPLC separations. Group separations and low-temperature separation of thermally sensitive compounds such as mono-, di-, triglycerides and free fatty acids will illustrate comparisons with GC. The practical ease of peak fraction collection using carbon dioxide and packed columns will be demonstrated and discussed.

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CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY. M. L. Lee, Brigham Young University, Department of Chemistry, Provo, UT 84602.

Theoretical calculations have indicated that supercritical fluid chromatography (SFC) using open-tubular (capillary) columns is feasible when columns less than 100 μm inner diameter (ID) are used. These theoretical predictions have recently been verified by constructing HETP vs mobile phase velocity curves for various solutes chromatographed under SFC conditions. Typically, efficiencies greater than 3,000 plates/m are obtained using columns of 50 μm ID. Columns with as low as 25 μm ID have been evaluated. Fortunately, thicker stationary phase films are allowable (minimal decrease in efficiency) in SFC compared with gas chromatography. This permits greater sample loads with increased detectability. Many technical problems associated with the preparation of small-diameter capillary columns with thick films have been overcome. Free-radical crosslinking was found to be essential to render the stationary phase insoluble in the various supercritical fluids used as mobile phases. The construction of instrumentation for capillary SFC has been rather straightforward. A high-pressure syringe pump, constant temperature oven, and any of the common liquid chromatographic (UV absorption and fluorescence) or gas chromatographic (FID, NPD, and FPD) detectors or mass spectrometry can be used. The use of a microcomputer for pressure (or density) programming, as well as for spectral scanning has proven to be invaluable. With the present instrumentation, column technology, and usual mobile phases (i.e., carbon dioxide, nitrous oxide, and n-pentane), relatively nonpolar thermally labile and high molecular weight compounds have been chromatographed. Polystyrene oligomers up to nearly 4,000 Daltons have been resolved using n-pentane as the mobile phase. The most pressing area of research today in capillary SFC is the development of mobile phase/stationary phase combinations for the analysis of more polar solutes. New capillary column surface deactivations and more polar mobile phases are presently under study. The success of research in this area will largely determine the ultimate potential of this technique for broad-spectrum range analyses.

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ANALYTICAL APPLICATIONS OF SUPERCRITICAL FLUIDS: CAPILLARY COLUMN CHROMATOGRAPHY AND MASS SPECTROMETRY. R. D. Smith, B. W. Wright, C. R. Yonker and H. R. Udseth, Battelle Pacific Northwest Laboratories, Chemical Methods and Kinetics Section, Richland, VA 99352.

Supercritical fluids have a wide range of potentially important applications in analytical chemistry. By increasing the pressure of the supercritical fluid, its properties may be varied continuously from those of a gas to those of a dense fluid with the solvating power of a liquid. The potential for improved chromatographic methods has been recognized for 2 decades but only recently, with the development of capillary columns suited for supercritical fluid chromatography (SFC), has this potential begun to be realized. Capillary columns of 35 to 75 μm inner diameter (ID) are presently being used for a range of fluids and fluid mixtures and are well suited to the variation of mobile phase solvating power by pressure (density) programming. One of the more powerful detectors for capillary SFC being investigated is the mass spectrometer. Supercritical fluids offer distinct advantages for the transport of thermally labile compounds to the mass spectrometer ion source. The development of the direct fluid injection (DFI) mass spectrometer interface in our laboratory has resulted in methods for the direct characterization of supercritical fluid extractions (and related fluid phase phenomena) as well as a combined capillary column SFC-MS approach. This approach allows thermally labile and high molecular-weight compounds to be separated and analyzed with detection limits in the picogram range. In this presentation, the fundamental aspects of SFC-MS will be reviewed and recent work aimed at the development of new detectors for SFC will be discussed. Examples of applications will include mycotoxins of the trichothecene group. The present limitations of the methods and new developments for extension to polar systems and high molecular mixtures will be described.

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SFC/FTIR: DEVELOPMENT AND APPLICATIONS. K. H. Shafer and P. R. Griffiths, University of California-Riverside, Department of Chemistry, Riverside, CA 92521.

Supercritical fluid chromatography (SFC) interfaces with Fourier transform infrared (FTIR) spectrometry by using a flow cell and a diffuse-reflectance accessory. The advantages and disadvantages of chromatography, sensitivity, and spectral information content of each interface is discussed. Detection by ultraviolet (UV) and flame ionization detectors (FID) are shown with identifications made by on-line FTIR spectroscopy. The IR transparency of CO_2 is examined with changing pressure of the supercritical fluid. Separations are performed on wall-coated, open-tubular and microbore high performance liquid chromatography (HPLC) columns. A comparison is made between gas chromatography (GC) and HPLC columns concerning column capacity, separation efficiency, and speed of analysis for SFC/FTIR. A coal extract is analyzed by SFC/FTIR and the results demonstrate the analytical utility of this instrumental technique. Because of the functional group specificity of infrared spectroscopy, the structural elucidation of high molecular weight, multifunctional compounds is nicely accomplished by SFC/FTIR and shown to be complementary to SFC/MS.

Session FF New Developments in Anti-Nutritional Factors—Others Wednesday p.m.

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AFLATOXIN CONTENT OF PEANUT HULLS. Timothy H. Sanders and Jack L. McMeans, USDA, ARS, National Peanut Research Lab., 600 Forrester Drive, Dawson, GA 31742.

The potential for aflatoxin contamination in peanut hulls was determined by analyzing hulls from 20 commercial peanut lots known to contain aflatoxin (13–353 ppb). Hulls from all of the machine-shelled lots contained aflatoxin (1–100 ppb). Peanuts grown in drought-stress environments to induce high levels of aflatoxin contamination were machine-shelled with extreme care and hulls contained 0–25 ppmb. When hulls were screened through successively smaller round-hole screens, the aflatoxin content of the smallest fraction (8/64 fall through) was always highest and indicated that small peanut kernels and peanut parts actually contained

the aflatoxin. Hulls from the same environments obtained by hand-shelling did not contain aflatoxin. Hulls, containing no peanut kernels or parts, innoculated with a toxigenic strain of *A. flavus* and incubated for 10 days at 25 C contained no aflatoxin although growth was evident.

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ANALYSIS OF TRYPSIN INHIBITORS IN SOY PRODUCTS: EVALUATION OF METHODOLOGY AND IMPROVEMENTS. William L.Lehnhardt and Helen G. Dills, A. E. Staley Manufacturing Co., 2200 E. Eldorado, Decatur, IL 62525.

The method for determination of soybean trypsin inhibitors as described by Kakade et al. was evaluated for reagent stability, incubation parameters and the hydrolysis kinetics of the synthetic substrate N-benzoyl-D,L-arginine-p-nitroanalide (BAPNA). The BAPNA reagent decomposes slowly with time causing variations in the apparent trypsin inhibitor value. Preparation of the reagent from appropriate concentrates immediately before analysis eliminates this variable. The addition of calcium ions during the incubation of trypsin and potential inhibitor reduces the autolytic trypsin inactivation. Trypsin inactivation is more pronounced at alkaline pH regions. Phytic acid produces an apparent inhibition of trypsin when insufficient calcium ions are present. The addition of 25 mM calcium ion to the incubation system reduces both trypsin inactivation and the effects of phytic acid. The proposed modifications stabilize the trypsin activity and extend the linearity of the trypsin inhibitor determination.

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ARE TOASTED SOYBEAN FLOUR PROTEINS RESPONSIBLE FOR TRYPSIN INHIBITOR ACTIVITY? David J. Sessa, Northern Regional Research Center, 1815 N. University Street, Peoria, IL 61604.

Compounds in toasted soybean flour that possess trypsin inhibitor (TI) activity were isolated and characterized. TI activity was assessed by quantitating the inhibition of trypsin hydrolysis of N-benzoyl-DL-arginine-*p*-nitroanilide. Sodium hydroxide (0.01 N) extracts of toasted soybean flour had an average of 2.59 mg TI/g sample. These extracts, treated with trichloracetic acid (TCA) adjusted to 0.8 N in the reaction mixture, yielded, after extensive dialysis, a supernatant fraction that had 0.51 mg TI and a precipitate with 23.25 mg TI. The addition of polyvinylpyrrolidone to eliminate any tannins and phenolics in the base extracts that may interfere with the assay by giving an overestimation of TI activity, was deemed unnecessary. Material balance studies showed a total of 91% recovery of protein and 92% recovery of TI activity in both the TCA supernatant (1.1% protein; 2.0% TI) and precipitate (89.8% protein; 89.8% TI) fractions. Based on analysis by column chromatography and electrophoresis, the TCA supernatant and precipitate fractions contained mainly proteins, which were responsible for the TI activity.

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PROTEOLYSIS OF TRYPSIN INHIBITORS DURING THE GERMINATION OF LEGUME SEEDS. Karl A. Wilson and Anna L. Tan-Wilson, Department of Biological Sciences, State University of New York at Binghamton, Binghamton, NY 13901.

In the mung bean (*Vigna radiata*), trypsin inhibitory activity remains relatively constant during the first 22–3 days of germination, and rapidly declines thereafter. Examination of the mung-bean trypsin inhibitor (MBTI) by a combination of separation techniques reveals a rapid conversion of the inhibitor present in the ungerminated seed (MBTI-F) to a series of new inhibitor forms: F → E → C → A. Amino acid sequence analysis shows that MBTI-F is a typical Bowman-Birk type trypsin inhibitor of 80 amino-acid residues. Inhibitor E is produced from F by the removal of the carboxyl-terminal residues 77-80, whereas the further loss of residues 76-77, residues 1-8, and an internal cleavage at Ala^{35}-Asp^{36} produces inhibitor C. Using an assay based on polyacrylamide gel electrophoresis, the activity catalyzing the conversion of inhibitor F to E at a pH optimum of 4.0 was found to be present in ungerminated seeds. It is unaffected by phenylmethylsulfonyl fluoride (an inhibitor of plant carboxypeptidases), iodoacetate and leupeptin (both inhibitors of the major sulfhydryl-dependent proteinase of germinated mung beans), and also pepstatin, chymostatin and antipain. After 1 day of germination activity, converting inhibitor E to C is present and increases until at least day 6. This activity is inhibited by phenylmethylsulfonyl fluoride, but unaffected by the other inhibitors tested. The enzyme catalyzing this apparent conversion of MBTI-E to -C has been purified from the cotyledons of seeds germinated for 6 days by ion exchange chromatography and gel filtration. It has a molecular weight of ca. 60,000, and is active against MBTI-E, but has no effect on MBTI-F or -C. It readily hydrolyzes Cbz-Phe-Ala, Cbz-Phe-Leu, and Cbz-Ser-Met, and is inhibited by phenylmethylsulfonyl fluoride. This enzyme may account for some of the carboxypeptidase activity previously noted in germinating mung-bean seeds.

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ENZYME INHIBITORS IN DRY BEANS. S. K. Sathe, Department of Nutrition and Food Science, Muscle Biology Group, University of Arizona, Tucson, AZ 85721, and S. S. Deshpande, University of Illinois, Urbana, IL 61801.

Although dry beans are an important source of proteins and carbohydrates, they also contain several undesirable components, e.g., enzyme inhibitors, phytates, flatus factors, tannins, beany flavors and lectins that may impede their being used to full potential. The types of enzyme inhibitors present in legumes include trypsin, chymotrypsin, amylase, pectinase, cellulase, lipase, galactosidase and subtilisin and are both proteinaceous and nonprotein in nature. These enzyme inhibitors are of concern as they may cause underuse of corresponding substrate(s), thus possibly lowering the nutritive value of beans and bean products as a food source for humans and animals. In this paper, recent progress made in understanding these enzyme inhibitors will be reviewed.

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CHARACTERISICS OF α-AMYLASE INHIBITORS IN *Phaseolus vulgaris* BIOTYPES. R. Hoover and F. Sosulski, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

Flours from 6 legume species (lentil, lima bean, field pea, chickpea, fababean and mung bean) were devoid of α-amylase inhibitory activity but 5 biotypes of *Phaseolus vulgaris* showed activities of 27–40 units/mg protein. Air classification of the pin-milled flours yielded fine fractions that contained 37% (black bean) to 61% (navy bean) protein and 38–66 units of inhibitor activity/mg protein, respectively. The coarse starch fractions were proportionately depleted in protein content and inhibitor activity. The partially purified amylase inhibitor from navy bean was active toward porcine pancreatic α-amylase. The optimum pH for inhibition was 5.7, with the inhibitor being most stable at pH 6.9 after 4 hr incubation at 37 C. The inhibition was faster at 37 C than at 25 C. Although retaining most of its inhibitory power between 37–60 C, the navy-bean inhibitor showed a complete loss of activity after 20 min at 90 C.

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THE BIOLOGICAL UTILIZATION OF SULFUR AMINO ACID ISOMERS AND DERIVATIVES. Mendel Friedman and Michael R. Gumbmann, Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, CA 94710.

L-amino acid residues in food proteins are subject to racemization to D-isomers under food-processing conditions. Because D-amino acids and L-amino acid derivatives may become part of our diet, a need exists to assess the factors that influence their formation, biological use and safety. In this presentation, we report on 14-day growth assay studies on a synthetic amino-acid diet fed to mice in which sulfur amino-acid isomers and derivatives were partly or fully substituted for L-methionine. Substituting D-methionine for the L-isomer resulted in a dose-dependent weight gain ranging from 66 to 94% of corresponding values obtained with L-methionine. L-cysteine and derivatives stimulated weight gain in the presence of suboptimal (1/4 of maximum or 0.293% in the diet) levels of L-methionine. Weight gain with suboptimal L-

methionine was 7.4 ± 0.7 g. With N-acetyl-L-cysteine, the weight gain was 214% of the value observed with L-methionine alone; with L-cysteine, 178%; with L-cysteic acid, 154%; and with (DL + meso)-lanthionine, 127%. D-cysteine and D-cystine, however, produced weight losses. All compounds, except D-cysteine and D-cystine, therefore, appeared to have a nutritionally sparing effect on L-methionine. The observed growth-depressing effect implies that these 2 D-sulfur amino acids impose a metabolic burden and may be toxic at some concentration levels. These results will be interpreted in terms of known and postulated transamination and transsulfuration pathways governing the catabolism and metabolism of sulfur amino acids.

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DRY BEAN TANNINS: NUTRITIONAL IMPLICATIONS. N. R. Reddy, Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, S. K. Sathe, Department of Nutrition and Food Science, University of Arizona, Tucson, AZ 85721, and D. K. Salunkhe, MPAU, Rahuri 413722, Maharashtra State, India.

Dry beans are an important staple in the diets of people in many areas of the world. Tannins are one of the several antinutritional factors present in dry beans and are mainly located in the seed coat or testa. Tannin content of dry beans ranges from 0.0% to 2.0%, depending on the species. Naturally occurring food tannins are known to interact with proteins (both enzymatic and nonenzymatic) to form tannin-protein complexes leading to the inactivation of digestive enzymes (trypsin and α-amylase) and making proteins insoluble. Both in vitro and in vivo studies indicate that bean tannins decrease protein digestibility, possibly by either inactivating (at least partially) digestive enzymes or reducing the susceptibility of the substrate proteins after forming complexes with tannins and ionizable iron absorption. Excessive intakes of tannins in the diet are also reported to cause certain undesirable ultrastructural changes in the digestive tract linings in the experimental animals. The antinutritional activity of bean tannins can be partially or completely reduced by several processing methods (1 or combination of 2 or more), e.g., dehulling, soaking, cooking and germination. Genetic selection may also help in breeding varieties low in tannins. Possible use of chemical treatment such as tannin-complexing agents will be discussed.

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RECENT DEVELOPMENTS IN THE ASSAY AND CHARACTERIZATION OF TANNIN IN SORGHUM GRAIN. Larry G. Butler, Department of Biochemistry, Purdue University, West Lafayette, IN 47907.

Sorghum grain contains from 0% to 2% condensed tannin (oroanthocyanidin, oligomers of flavan-3-ols). High-tannin sorghums are somewhat resistant to depredation by birds and fungi, but are nutritionally inferior to low-tannin sorghums. Sorghums have been classified into 3 groups according to the amount of tannin they contain and the conditions required for its extraction; these groups are morphologically and genetically distinct. The 2 groups that contain significant levels of tannin have been examined for differences in biological activity such as bird resistance and for differences in chemical structure at several stages of maturity. The results suggest that the tannin from mature sorghum grain, which has been purified and chemically characterized, may not be the polyphenol component most biologically active.

Session GG New Crops and Processes Wednesday p.m.

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TEXTURED PEANUT PROTEIN: A RESPONSE SURFACE METHODOLOGY. E. Trejo, E. Ríos, M. Hernández and J. L. Camacho, Instituto Nacional de la Nutrición "Salvador Zubiran," Departmento Ciencia y Tecnología de Alimentos, Vasco de Quiroga N°15, Col. y Deleg. Tlalpan, C.P. 14000, México, D.F., México.

Defatted peanut flour texturization was carried out, using a Wenger X-5 laboratory extruder, in order to evaluate the best extrusion conditions to obtain textured peanut protein (TPP). The best extrusion conditions were evaluated by response surface methodology (RSM). Functional characteristics studied were: water absorption index (WAI); fat absorption index (FAI); emulsification capacity (EC); nitrogen solubility index (NSI). In the case of NSI, enough evidence was found to minimize this variable. NSI results analyzed by RSM showed that optimal conditions of the extruder to obtain the lowest NSI value are 29% humidity in the peanut flour, 115 C in the last phase of texturization and a screw rotational speed of 530 rpm. The resultant TPP presented an 8% moisture content, 54.1% protein, 5.8% NSI, 3.6 g gel/g dry sample for WAI, 93.1 mL oil/100 g sample (with 14% moisture) as FAI and a EC value of 80.6 mL of oil/100 mg protein (dry basis). The fact that the remaining variables did not show extrusion conditions to establish the maximum or minimum values in accord with Taylor's equation can be imputed to the presence of sodium stearoyl-2-lactylate that was mixed with the peanut flour, because this compound modifies the rheological characteristics of textured products.

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PARTIALLY DEFATTED PEANUTS: COST ANALYSIS. K. M. Decossas and J. Pominski, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Partially defatted peanuts prepared by a simple, economical process have chemical and physical characteristics that make them attractive for use in a variety of tasty and nutritious foods and confections. They contain less than half the native oil of full-fat peanuts, a substantially higher 43 wt-% protein, and notably fewer calories. They also have the highly desirable characteristic peanut flavor and texture. A new, highly efficient, continuous process is described including novel systems for bulk handling of peanuts, salt transfer, cornstarch unloading–storage-transfer, and the membrane processing of expansion water effluent for its reuse and for recovery of soluble sugars and protein by-products. A material balance and flowsheets for batch and continuous processing are given, and hypothetical grass-roots plants are described for batch production of 2.6 tons daily and continuous production of 8, 40 and 80 tons daily. Capital costs, manufacturing costs, general expenses, profitability and selling prices are indicated for production of up to 20,000 tons annually of the hypothetical plants.

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PROCESSING OF *Crambe abyssinica* SEED IN COMMERCIAL OILSEED EXTRACTION FACILITIES. K. D. Carlson, E. C. Baker and G. C. Mustakas, Northern Regional Research Center, 1815 N. University Street, Peoria, IL.

Oil extraction and production of defatted meal from *Crambe abyssinica* were studied in 4 different commercial extraction facilities involving 6 distinct runs or processing segments. These plants were set up for either prepress solvent extraction or straight solvent extraction techniques, using a variety of equipment for seed preparation, pressing and flaking, extracting, and desolventizing and toasting. Plant capacities ranged from several t/day to 200 t/day, with processing of crambe in the larger facilities generally ranging from 50 to 100 t/day to provide meaningful runs suited to the quantity of seed available. Purposes of the studies were to demonstrate the commercial feasibility of processing crambe along lines developed in numerous laboratory and pilot-plant studies, and to prepare quantities of defatted meal for beef cattle feeding studies. The factors evaluated were seed storage, cleaning and preparation for oil extraction, expelling or pressing and flaking steps, solvent extraction phases, and desolventizing and toasting operations. Primary concerns during the runs were thioglucosidase enzyme inactivation, preventing or minimizing glucosinolate degradation to toxic aglucon products in the finished meals, oil extraction efficiency, and quality of oil and meal products. Onsite testing included measurements of moisture, oil, glucosinolate, and temperature and estimates of enzyme activity. Many grab samples of seeds, press cake or flakes, and meals, as well as composited samples from 8- to 16-hr

"steady state" sequences, were collected. The studies indicate that processing in a suitably equipped plant should offer no problems to commercialization of crambe for oil recovery or feed production. As reported earlier, crambe meal already has FDA approval for use as a feed supplement up to 4-1/2% of the total ration.

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INEDIBLE RENDERING SYSTEMS WITH REDUCED ENERGY USE. William H. Prokop, National Renderers Association, Inc., 3150 Des Plaines Avenue, Des Plaines, IL 60018.

During the past 5 years, new rendering systems have been developed to use the vapors from a continuous drying or cooking operation to obtain further moisture removal. Most of this new rendering technology has evolved in Europe, where energy costs have been significantly higher than in the United States. In these systems, the raw material is ground, preheated and then subjected to a pressing operation for separation into 2 phases, a presscake of solids containing fat and moisture and a liquid containing melted fat and water. The solids are passed through a drying or cooking operation conducted at atmosphere to evaporate the moisture. The waste heat in the vapors from the dryer or cooker are used to evaporate the moisture from the liquid removed by pressing the raw material. This second stage of evaporation is normally performed under vacuum. Several rendering systems are described to illustrate this principle of reduced energy use. These systems are operated at lower evaporation temperatures than those used by conventional rendering systems. Also, specific performance results are presented to illustrate the amount of reduced steam usage and improvement in product quality obtained with these systems.

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PREPARATION, COMPOSITION, AND UTILIZATION OF RICE-BRAN OIL, AND ITS POTENTIAL EXPLOITATION FROM STABILIZED RICE BRAN. R. M. Saunders and R. N. Sayre, Western Regional Research Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710.

The key to economic recovery of edible rice-bran oil is to extract the oil before appreciable free fatty acid (FFA) development. This is normally practiced in a limited way, by extracting the bran immediately after milling, or during milling (X-M process). Alternatively, and logistically more appropriate, the bran could be stabilized immediately after milling. Then the bran could be accumulated over time before extraction. Stabilization arrests FFA development by destroying lipolytic enzymes. Stabilization is not currently practiced anywhere in the world, although recent results in the US would appear to presage the use of low-cost extruders to meet this requirement. Rice-bran stabilization, oil refining, oil composition and uses of industrial and edible grade oils will be described. Rice-germ oil will also be described.

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EXTRACTION OF RUBBER FROM GUAYULE. Cady R. Engler and Timothy J. Smith, Food Protein Research and Development, F.M. Box 183, Texas A&M University, College Station, TX 77843-2476.

Guayule (*Parthenium argentatum* Gray) is a shrub native to semi-arid regions of Texas and northern Mexico that contains an appreciable amount of natural rubber. Molecular structure and properties of rubber from guayule are nearly identical to natural rubber produced commercially from rubber trees (*Hevea brasiliensis*). However, unlike *Hevea*, in which rubber latex is contained in ducts, guayule rubber is contained in latex particles distributed throughout plant cells. To free guayule latex for extraction, cell structures must be disrupted, which allows resins and other contaminants to mix with the rubber. Therefore, additional purification steps for separation of resins and particulates are required to produce rubber equivalent to *Hevea*. Natural rubber from guayule was produced intermittently on a commercial scale during the first half of this century, using a wet milling process followed by flotation to separate rubber from bagasse. Major efforts to improve the flotation process occurred as part of the Emergency Rubber Project during World War II, and process development continued at the US Natural Rubber Research Station in Salinas, California, until mid-1953 when funding of the project was terminated. In 1976, the Mexican government began operation of a pilot plant, integrating technology from the pulp and paper and synthetic rubber industries into the flotation process. Because of the large water requirements for flotation processes, and the need to use solvents for purification steps, recent efforts in the US have been directed toward development of solvent extraction processes. Two general approaches have been considered: (a) extraction of resins with a polar solvent (acetones or alcohols), followed by extraction of rubber with a hydrocarbon solvent and (b) simultaneous extraction of rubber and resins with a hydrocarbon solvent, followed by separation of resin and rubber fractions. A pilot plant, based on the simultaneous extraction approach, has been operating for several months to produce quantities of rubber for product testing.

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NEW DEVELOPMENTS IN NONCALORIC (CALO) OILS AND FATS. Melvin Wolkstein, Reach Associates, Inc., 75 South Orange Avenue, South Orange, NJ 07079.

The world research activity on low caloric, nonmetabolizable fats since Deuel, Cheng and Morehouse (J. Nutrition 35:295, 1948) has not been accompanied by many publications. The international patent literature provides a panorama of activities on such fat substitutes, which we call CALO fats and oils. Sixty-two first filings of patents deal with sucrose esters and sucrose ester alcohols alone pioneered by Proctor & Gamble 15 years ago. Pharmaceutical patents have been obtained alongside the edible ones for cholesterol and arteriosclerosis. It has been estimated that before the end of the century, more than 5% of the current 60 million tons of edible fats in the world will be of the CALO type, necessitating an unprecendented expansion of the fatty acid, mannitol and similar businesses. Overall, oil and fat research in the framework of CALO products is placed in evidence in the paper, necessitating capacity increases and new large-scale synthesis. Among new developments discussed are the use of CALO fats in partial substitution of butter fat in ice cream and frozen desserts, along with the use of aspartame non-caloric sweeteners (Dairy Field, November, 1983) and tofu frozen desserts, which now use soybean oil in soft-serve and soon in hard-frozen composition.

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MANUFACTURING OF PLANT EXTRACTIVES, THE STATE OF THE ART TECHNOLOGIES. Joaquin Pelaez, McCormick & Company, Inc., 202 Wight Avenue, Hunt Valley, MD 21031.

The majority of plant extractives have always been achieved by steam distillation. However, other techniques such as extraction with volatile solvents and expression have also been used. New extraction technologies such as Liquid CO_2 and Supercritical CO_2 are being investigated for the manufacturing of these extractives. This paper will review the advantages and disadvantages of all these methods.

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MODERN FISH OIL AND MEAL PROCESSING. Adne Ø Utvik, Stord Bartz a.s., C. Studtsgate 29, P.O. Box 777,5001, Bergen, Norway.

A summary of some low-energy rendering processes is presented with special emphasis on energy-saving techniques introduced in fish-meal manufacturing in recent years. This technique has cut fuel consumption dramatically and improved product quality.

Session HH Color Problems: Science and Technology

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THE HISTORY, PRESENT STATUS AND FUTURE USES OF FOOD COLOR MEASUREMENTS. Richard S. Hunter, Hunter Associates Laboratory, Inc., 11495 Sunset Hills Road, Reston, VA 22090.

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In the food industry, color measurements are applied to transparent liquids (oils, beverages and so forth), to opaque diffusely reflecting surfaces (tomato and other vegetable products), and to translucent substances that are optically intermediate between opaque and transparent. Color measurements of transparent edible oils by visual comparisons of sample with standards date from about 1880. Color measurements of opaque tomato and other products by photoelectric sensing and CIE conversions to tristimulus values of color date from ca. 1930. A variety of procedures have been proposed for the measurement of colors of translucent food substances. The first objective of the current project is to simulate one of the most popular visual methods still in use (Lovibond) by photoelectric scanning and microprocessor computation. The change should improve both the speed and reproducibility of color measurements without serious departures from the older numerical Lovibond scales with which technologies in the edible oil industry are familiar. In the future, one can expect instrumental measurements of the color of transparent substances in 3 dimensions (rather than one) and the addition of measurements of geometric light distributions for haze, turbidity, texture and so forth. Many of these measurements will be made on-line rather than in the laboratory.

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CHARACTERISTICS AND FOOD APPLICATIONS OF COMMERCIALLY AVAILABLE SYNTHETIC CAROTENOIDS. Howard T. Gordon, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110.

The first total synthesis of pure β-carotene was achieved in 1950. Since then, 3 carotenoids have been approved for food use in the US: β-carotene, β-apo-8'-carotenal and canthaxanthin. These compounds and the physical and chemical characteristics of the numerous market forms in which they are available will be reviewed. Special emphasis will be given to the application of these products in oil-based foods, including stability, variations in color hue in finished foods, labeling considerations and nutritional contribution.

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A CHLOROPHYLL SELECTIVE BLEACHING CLAY: CONTRAST IN CHLOROPHYLL REMOVAL FROM SOY AND CANOLA OIL. Dennis R. Taylor and Charles B. Ungermann, Kaiser Aluminum & Chemical, P.O. Box 877, Pleasanton, CA 94566.

The major objective of using bleaching clays in the processing of vegetable oils is to remove colored pigments, including carotenoids and chlorophyll. Quite often, particularly when green oils obtained from green or immature beans must be processed, the emphasis is on removing chlorophyll. Although the overall decolorizing activity of bleaching clays can be increased, because carotenoid reduction is usually quite adequate for modern high-activity clays, what is really desired is selectivity for chlorophyll removal. Using modified bleaching clays, to effect the more selective removal of chlorophyll from soybean oil has been possible. In contrast, no selectivity effect was observed for canola oil. This disparity in results is discussed in terms of differences in carotenoid composition and chlorophyll levels between these 2 oils. Some selected work with chlorophyll removal from sunflower oil is also reported.

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EFFECT OF COLOR ON FLAVOR JUDGMENTS OF FOOD. Carol M. Christensen, Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104.

The color of food is a significant element in its acceptance by consumers. Yet little is understood of the factors contributing to its importance; in particular, the role of food color in the perception of aroma and taste. This paper reviews research, including the author's, of the effect on food-flavor perception of the presence or absence of food colors or manipulations of food-color strength. The findings discussed include the effects of food color on the ability to identify flavors, judge flavor strength and discriminate foods of varying flavor strength. In general, researchers find that absence of food colors significantly impairs flavor identification and reduces the perceived intensity of flavors. Adjustments of color strength have little effect on the subjects' judgments of flavor.

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PIGMENTS AND PIGMENT-ARTIFACTS IN COTTONSEED. Alois A. Bell and R. D. Stipanovic, USDA, ARS, National Cotton Pathological Research Laboratory, P.O. Drawer JF, College Station, TX 77841.

Oil and flour products prepared from cottonseed often show undesirable yellow and brown colors. The main compounds contributing yellow colors are terpenoid aldehydes and flavonol glucosides. The terpenoid aldehydes are localized in lysigenous glands found mostly in the cotyledons of the seed embryo. In Upland cotton (*Gossypium hirsutum* L.), the only appreciable terpenoid is gossypol, which generally makes up 0.3 - 1.0% of the seed embryo. In Pima cotton (*Gossypium barbadense* L.), mono- and dimethyl ethers of gossypol make up 10- 30% of the total terpenoid aldehydes. Concentrations of terpenoid aldehydes are determined by reactions with aniline or phloroglucinol. Oxidation products of hydroxylated unsaturated fatty acid triglycerides in cotton and okra seed also react with these reagents to give false positive readings. Such reactions may account for some reports of low gossypol concentrations in glandless cotton and okra seed. Brown pigments apparently are melanoid pigments formed by oxidation of condensed tannins. Ca. 10% of the seed coat is tannin or melanin, and considerable amounts also occur in the nucellus surrounding the seed embryo. Only a few scattered cells of the seed embryo contain tannins.

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DISCOLORATION IN FOOD PRODUCTS SUPPLEMENTED WITH EDIBLE COTTONSEED FLOURS. Florine A. Blouin and Zigrida M. Zarins, USDA, Agricultural Research Service, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Edible cottonseed flours are protein products that can be used as ingredients in foods to add nutritional value or to introduce new functional properties. When glanded or glandless cottonseed flours are used in baked products as a replacement for wheat flour at the 20% level, they cause a yellow-brown discoloration that could adversely affect consumer acceptability of the product. The yellow color is caused by flavonoids that are present in both types of cottonseed flour. These flavonoids were isolated and shown to be glycosides of quercetin and kaempferol. They are easily removed by aqueous alcohol extraction. Glanded cottonseed flour causes a dark-brown discoloration whereas glandless flour causes a light-brown discoloration. The brown color-causing components are not readily removed from the flours. A series of fractionation steps using solvent extractions and enzyme digestions was devised to remove various other components and thereby concentrate the color-causing components for characterization. The dark brown color-causing components in glanded cottonseed flour were shown to be gossypol derivatives bound to lipoprotein fractions of the flour. The light brown color-causing components in glandless flour, also present in glanded flour, are part of the most insoluble flour fractions and are thought to be phenolic components of the tannin or lignin type.

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A STUDY OF THE CAUSE OF RAPID COLOR DEVELOPMENT OF HEATED REFINED PALM OIL. Y. A. Tan and S. H. Ong, Palm Oil Research Institute of Malaysia, 18th Floor, Angkasa Raya, Jalan Ampang, Peti Surat 620, Kuala Lumpur, 04-06, Malaysia.

One of the most obvious changes when oils are heated is color darkening. Palm oil darkens very rapidly compared with other oils. The cause of this rapid color development was investigated. Various methods were used to pretreat Lotox crude palm oil (CPO) to retard darkening during heating. The 3 treatments were: agitation with activated carbon S511; water and water isopropyl alcohol (95:5) washing of neutralized and unneutralized oil; and liquid/liquid extraction of oil using water and water and isopropyl (95:5). Both treated and untreated oils were then bleached by the SCOPA method before being subjected to heat at 180 ± 5 C for 49 hours (7 hours daily). Analyses showed that pretreatment of CPO did

succeed in retarding color development. Retardation was especially evident in oils previously neutralized with sodium hydroxide before washing with water and water and isopropyl alcohol. The light yellow aqueous extracts from the liquid/liquid extraction process were subsequently analyzed to identify the color-causing components of CPO. The UV spectra of the extracts showed strong absorption maxima at 256 nm. The addition of a base resulted in the darkening of the extracts accompanied by shifts to longer wavelengths (288 nm). Reaction with freshly diluted 1–2% ferric chloride solution gave a brown color. The development of paper chromatography in butanol:acetic acid:water (6:1:2) revealed a blue fluorescence near the solvent front, with the same relative retention time as that of tannic acid. This evidence indicates that phenolic compounds were responsible for color darkening in palm oil.

Session II Analytical Chemistry (General) Wednesday p.m.

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LIPID QUALITY CHROMATOGRAPHICALLY DEFINED AND MEASURED BY NEAR INFRARED TECHNIQUES. D. L. Wetzel, Grain Science Department, Kansas State University, Manhattan, KS 66506.

Lipid blends with varying properties were characterized by high performance liquid chromatography and gas chromatography to establish quality-related reference data for regression with spectroscopic data of the near infrared region. Data was obtained with a Technicon Infraalyzer 500 equipped with a thermostated liquid cell. Statistically, wavelengths were chosen and coefficients established relating near infrared measurements to lipid-quality factors defined by chromatographic results.

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DETERMINATION OF VOLATILE SULFUR COMPOUNDS IN CANOLA OIL. V. Abraham and J. M. deMan, Department of Food Science, University of Guelph, Guelph, Ontario N1G 2A1, Canada.

A simple and sensitive method for the quantitative determination of volatile isothiocyanates in Canola oil has been developed. The method is based on the specific absorbance of isothiocyanates in the infrared region. The results obtained were confirmed by gas liquid chromatography (GLC) using a flame photometric detector. The various volatile isothiocyanates isolated from the oil were allyl isothiocyanate, 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate and 2-phenethyl isothiocyanate. Their identities were confirmed by mass spectroscopy and by retention times. The recoveries of volatile sulfur by this method ranged from 93.6–101.11% when compared with the amount determined by GLC. The coefficients of variability of volatile sulfur to Canola oils ranged from 1.7–2.9%.

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MOISTURE DETERMINATION IN OILS AND FATS. T. Fossum, M. Kutter, Mettler Instrument Corporation, Box 71, Hightstown, NJ 08520, and K. Mooibroek, Mettler Instrumente AG, CH-8606 Greifensee, Switzerland.

Accurate moisture determination is a requirement for quality in food processing. The usual and widespread method for this determination is the Karl Fischer titration. The moisture content in fats can vary greatly, e.g., from 100 ppm in beef tallow to 88% in milk. Because of this broad range, various approaches are used for the water determination. The new Mettler KF Autotitrator is effective in the entire range of water content determination down to 1 ppm. This newly developed instrument allows automatic titration of available water in as little as 1 minute. Water content of a broad spectrum of samples has been analyzed with the new Mettler KF Autotitrator–the DL18. Fats and oils, e.g., sunflower oil, beef tallow, margarine, mayonnaise, butter, cream, cheese and milk, were evaluated. Results of solvent, titrant, temperature and stirring rate and time are discussed. The handling of low water samples is described and the evaluation, including automatic blank compensation and drift correction is discussed. The capabilities of this compact instrument to deliver precise and accurate results are demonstrated.

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A NEW RAPID METHOD OF DETERMINING PHOSPHORUS LEVELS IN VEGETABLE OILS. Ben Berck, Foods and Nutrition Department, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

For industrial monitoring of total P levels in vegetable oils, the official AOCS, Oxygen Bomb and various other analytical methods are slow and cumbersome for multiple determinations, and are inaccurate below 3 ppm P. A new, accelerated method developed here enables accurate assays in the range 0–800 ppm P of 3 preweighed samples within 1½ hrs and of 40 samples within 3 hrs. Screw-cap borosilicate glass tubes were used to contain 25–600 mg of sample, the amount depending on the type of oil or fat. To avoid time-consuming transfers, the oxidation, hydrolysis and color development steps were sequentially conducted in the same tubes. Ashing duration at 600 C ranged from 25 min/3 samples to 75 min/40 samples ashed at one time, followed by hydrolysis with 0.5 N HCl at 100 C/15 min. Colors were developed with mixed molybdate solution and were incubated at 50 C/15 min to accelerate color development. Absorbance (A) values were read at 820 nm. The mean A/μg ratio $\times$ 10^3 of the standard PO_4-P curve derived from 7 points in the working range 0.2–12.5 μg P was 77.6, and was highly reproducible. Standard deviations (SD) of 6 replicates of each of crude soy, degummed canola and refined canola oils of 578.43, 26.73 and 0.868 mean ppm P values were 3.697, 0.233 and 0.0157, corresponding to 0.64%, 0.87% and 1.80% relative SD. The new method was performance-tested against the AOCS method, and was considerably faster, simpler, more sensitive and reproducible, and more accurate in the 0–5 ppm range. Its fact-finding power in rapid P assays was explored with 42 diverse materials, including used frying oils, potato chips, peanut butter, lecithins, oilseed meal, bloodmeal, wash water, tap water, cheeses, Mt. St. Helens' volcanic dust, houseflies and so forth.

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QUANTITATIVE ANALYSIS OF THE TRIGLYCERIDE SPECIES OF VEGETABLE OILS. Fred C. Phillips, Warren L. Erdahl and Orville S. Privett, The Hormel Institute, University of Minnesota, 801 – 16th Avenue, N.E., Austin, MN 55912, and John A. Schmit, E.I. DuPont de Nemours & Company.

A method for the quantitative analysis of triglyceride species composition of vegetable oils by reversed-phase high performance liquid chromatography (RP-HPLC) via a flame ionization detector (FID) is described. Triglycerides are separated into molecular species via Zorbax chemically bonded oxtadecylsilane (ODS) columns using elution with methylene chloride in acetonitrile. The identification of species is made from reference compounds and the comparison of the experimental and calculated theoretical carbon numbers (TCN) relative to their abundance, determined by a random distribution of the major fatty acids. Quantitative analysis is based on a direct proportionality of peak areas. Differences in the response of individual triglyceride species were small and did not require the use of response factors. The method is applied to cocoa butter before and after randomization, soybean oil and pure and adulterated olive oil.

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ESTIMATION OF THE COMPOSITION OF EDIBLE OIL MIXTURES. P. J. Van Niekerk, A. E. C. Burger and W. H. van der Walt, National Food Research Institute, C.S.I.R., PO Box 395, Pretoria, 0001 South Africa.

The fatty acid, sterol and tocopherol contents of edible oils were used to determine the composition of oil mixtures by means of a weighted least squares estimator or a generalized least squares estimator with backward elimination. The models were tested on 30 binary mixtures of sunflower-seed, groundnut, cottonseed and soybean oils. The results of the weighted least squares model were closer to the real values than the results of the generalized least

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squares model. For the 30 mixtures, the differences between the percentage of composition of the major component as determined by the weighted least squares model and its true value were less than twice the standard error of the estimates. The standard errors ranged from 3% to 16%. Preliminary results indicate that the method could also be applied to 3 and 4 component mixtures.

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A RAPID HPLC METHOD FOR TOCOPHEROL ISOMERS. Chih-shang James Shen and A. J. Sheppard, Food and Drug Administration, 200 C Street, S.W., Washington, DC 20204.

A high performance liquid chromatographic (HPLC) system that uses a short column (100 mm × 4.6 mm) packed with 3-μm silica particles for separation of tocopherol isomers is reported. Baseline resolution of α, β, γ and δ tocopherols was obtained under isocratic condition using 2% isopropanol in hexane as the mobile phase at room temperature. Chromatographic separation of tocopherol isomers on this short, efficient silica column was compared with that obtained on conventional columns (250 mm and 300 mm) packed with either pellicular material or 5-μm silica particles. The results showed that the short column was equally effective in separating tocopherol isomers and reduced analysis time by more than 50% when compared with conventional columns.

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HPLC-MS-COMPUTER ANALYSIS OF LIPIDS. Warren L. Erdahl, Fred C. Phillips, Dale E. Jarvis and Orville S. Privett, The Hormel Institute, University of Minnesota, 801 16th Avenue, N.E., Austin, MN 55912.

The apparatus and techniques for the analysis of lipids by high performance liquid chromatography used in conjunction with computer-controlled chemical ionization-mass spectrometry are presented. The interfaces between the liquid chromatograph and the mass spectrometer and between the computer and the mass spectrometer are described. A rapid method for the quantitative analysis of volatile lipids is shown. The identification of lipid classes separated by adsorption chromatography and molecular species by reverse phase chromatography is also presented. The techniques are applied to reference compounds and to various examples of both plant and animal lipids.

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ANALYSIS OF ALKYNES AND ALKYNOIC ACIDS BY OZONE OR RUTHENIUM OXIDE OXIDATION. Leonard S. Silbert and Thomas A. Foglia, 600 East Mermaid Lane, Philadelphia, PA 19118.

Two analytical methods for determining the isomeric integrity and purity of the acetylenic bond in alkynes and alkynoic acids (esters) have been developed. One method is by ozonation and the other is by ruthenium oxide-catalyzed oxidation. Alkynes are rapidly ozonized (2–5 min) in methanol to carboxylic acids. The latter were esterified by the addition of BF_3 to the ozonized mixture and the methyl esters formed analyzed by capillary gas liquid chromatography. Ozonation to carboxylic acids were 90–99% complete with no attendant overoxidation to lower chain homologues. Coproducts, shown by partial ozonation reactions, were α-diketones, which in the esterification step formed dimethoxyketal ketones, identified by mass spectrometry. Both ruthenium tetroxide (RuO_4) and perruthenate anion (RuO_4^-) were examined for their utility in acetylenic bond determinations. RuO_4 overoxidized alkynes to lower acid homologues in addition to those expected under the conditions studied. On the other hand, RuO_4^-, in conjunction with a phase-transfer agent, oxidized alkynes to the expected acids but required longer reaction times (30–60 min) than ozonation. Over oxidation (<5%) was still a negative factor in quantitative triple bond determinations; however, RuO_4^- may be used for positional triple bond determinations. Moreover, it has the advantage over ozonation for aryl substituted alkyne analyses as ozone is reactive toward aromatic nuclei.

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THE DETERMINATION OF UNREACTED AMINES IN AMINE OXIDES BY POTENTIOMETRIC TITRATION. Chu Nan Wang and L. D. Metcalfe, Akzo Chemie, 8401 West 47th Street, McCook, IL 60525.

During the manufacture of amine oxides, information on the amount of unreacted tertiary amine present is needed in order to follow the reaction. A number of analytical procedures have been devised to obtain this information. Wet methods include redox titrations of the amine oxide and differential titrations using derivatization of the amine or amine oxide. Various chromatographic procedures using GC, TLC and HPLC have also been reported. All of these procedures have some limitations. A simple, rapid, quality-control procedure would be useful. A rapid nonaqueous titration procedure has been developed in our laboratory that makes use of the "anomalous salt" behavior of amine oxides. A modified solvent and titrant is used to obtain 2 potential breaks in the titration. The first break corresponds to half of the amine oxide. The second break represents the second half of the amine oxide plus any unreacted amine. With this information the amine oxide and unreacted amines can be calculated. The precision and accuracy of the titration has been studied using samples spiked with known amounts of amine.

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A NEW INSTRUMENT FOR THE CHARACTERIZATION OF MELTING AND CRYSTALLIZATION BEHAVIOR. M. Kutter, G. Paul, Mettler Instrument Corporation, Box 71, Highstown, NJ 08520, and P. Schauwecker, Mettler Instrumente AG, CH-8606 Greifensee, Switzerland.

The determination of thermal properties is an essential step in the quality control of oils and fats. Various standard methods have been developed, however, many are tedious and prone to error. To increase accuracy, a new system has been designed for the automated temperature determination of physical transitions. The Mettler FP800 Thermosystem, which consists of a central processor and 5 different modules, uses optical detection as well as differential scanning calorimetry (DSC) for transitional temperature determination. Fats and oils display complex melting behavior that can be effectively described by a complete DSC curve. Several methods have been established, providing a single temperature to characterize this melting behavior. By evaluating the DSC curve, additional information can be obtained such as melting range, liquid fraction or solid fat index. The comparison of thermal values such as melting, dropping, slip point are determined automatically by the FP800 system with the more empirical AOCS Official Methods will be given. The process of crystallization is described by the determination of the cloud point of oils and the congeal point of fats, respectively the titer of fatty acids. The advantage of the automatic determination in contrast to the visual observation will be demonstrated. The results of experiments with samples such as beef tallow, palm oil, butter, cocoa butter, margarine, vegetable oils and fatty acids will be discussed in terms of precision and accuracy.

Session JJ Computer Technology—Lab Applications Wednesday p.m.

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LABORATORY WORK STATION SYSTEM FOR PERSONAL COMPUTER. Loring A. Kutchins, Dynamic Solutions Corp., 61 South Lake Avenue, Pasadena, CA 91101.

Dynamic Solutions' current product, Appligration is an integrated hardware/software system for personal computers designed to serve a scientist in a research or testing laboratory in a variety of ways. Its first function is to serve as a data-acquisition system, taking information in the form of an analog voltage signal, converting it into a digital representation and storing it in the computer's memory. The Appligrator does this analog to digital conversion at rates ranging from once every 25 seconds up to 20,000 times per second on as many as 16 channels simultaneously. Once data is stored in memory, the software is designed to allow a wide variety of manipulations of the sampled waveforms. The scientist can

choose to have it graphically displayed on the video screen or dumped to a printer, and, subtract, divide or multiply any 2 such waveforms or do overlays and detect peaks in the waveform, thus yielding useful information in certain analytical applications. The entire raw data buffer can be stored on floppy disk or larger capacity Winchester hard derives for later retrieval and processing. Finally, the Appligration system can perform the above functions automatically. Included in the hardware is a 5-volt trigger to initiate data acquisition from an autosampler. This saves the researcher valuable time and allows him to concentrate on the end result, which might otherwise involve complex and tedious calculations. The concept of applications software is well known in the business market. These are end user, easy to use, well-documented software packages that perform all aspects of a broad task. In business, examples include word processing, accounting, inventory control and so forth. Such polished packages are remarkably lacking for scientific applications on microcomputers. This is the void that Dynamic Solutions has targeted to fill in the pharmaceutical, food, agricultural, environmental and industrial testing laboratories. The chromatography software package includes: gas, liquid, HPLC, GPC and amino-acid analysis. The other software packages include: spectroscopy (UV/VIS, NMR, FTIR, AAS), thermal analysis, velocimetry, continuous-flow colorimetry (Technicon auto analyzers, FIA). Report formats include: retention time, including peak start and end, area, area percentage, peak height percentage, area/ht ratio, response factor, ID, grouped peaks, normalization, molecular weights, molecular percentage, voltage and how the peak was integrated. Concentration reports include: external and internal standardization, linear and nonlinear, single- and multiple-point calibrations in significant compound reporting units. Report formats can be custom tailored by the user.

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Withdrawn

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COMPUTER-AIDED LABORATORY DATA MANAGEMENT. Walerian Kipiniak, Computer Inquiry Systems Inc., 160 Hopper Avenue, Waldwick, NJ 07463.

The talk will describe CIS' CALS Computer Automated Laboratory System with emphasis on its laboratory management functions. Implications of GLP on computer-aided laboratory record keeping will be discussed. Experience in configuring such computer systems to the requirements of QC and research and development laboratories will be presented.

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LABORATORY MANAGEMENT: THE GOAL OF LABORATORY COMPUTERIZATION. J. V. Hornstein, S. Chase and N. Birnbaum, Perkin Elmer Inc., Sobma Slavms, Norwalk, CT.

Analytical laboratories in the petroleum industry perform a large number of very diverse testing procedures and must correlate information from all these procedures to provide answers. The testing laboratories support services related to all aspects of the industry, from exploratory research to refinery operations to customer services. Within each laboratory, the tests range from manual measurements to particulate and suspended solids to very complex chromatographic separations and quantitations, to aid in process development via identification of components in complex reaction mixtures. Individually, each bit of information can answer only some portion of the overall question. The laboratory manager, or his delegate, must assemble the information, produce the report, and account for the use of time and materials within the laboratory. Computerization of the laboratory has both aided and hampered laboratory personnel. The coupling of data stations and chromatography automation systems to the instruments has reduced the burden on the chemist and technician. The data processing required for each analysis has been made very simple and very rapid. More information has been made available than ever before, at a lower cost per analysis. The development of the laboratory information management system, or LIMS, was undertaken to provide a way for the laboratory manager to assess the operation of the lab and provide a better service to the users. LIMS/2000 serves as a central repository for all the information related to a sample: results from all the tests, correlated data comparisons, and management information such as time per analysis, billing, equipment use and so on. LIMS/2000 can manage all the information required for laboratory operation, produce reports (both tabular and graphical) and issue bills, as well as collect and analyze data from a variety of analytical instruments. LIMS/2000 completes the job of laboratory computerization started by data stations, integrators, and automation systems.

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A NEW LABORATORY DATABASE SYSTEM FOR VAX-11 COMPUTERS. Arthur C. Brown, III, Varian Instrument Group, Walnut Creek, CA 94598.

Information management, laboratory automation and the resulting effects on lab productivity are important concepts that industry is coming to grips with now. An approach to solving these problems is a laboratory information management system (LIMS), based on the VAX family of 32-bit computers from Digital Equipment Corporation. The system is designed around a hierarchial or pyramid model of the lab with the instruments at the lowest level, data systems at the next level and the LIMS at the third level. This organization has several key benefits. First, it allows existing instruments and data systems to be used. Computer-compatible instruments can be connected directly to the LIMS for data transfers while older instruments can continue to be used with manual data entry through LIMS terminals. Second, the LIMS can serve several labs, groups or departments as a central computer resource. Information is stored in a centralized database for rapid and convenient access, and, computer programs and peripherals can be shared by all LIMS users. At the same time, the individual laboratories and departments retain their autonomy in selecting new instrumentation to fit their separate needs. Third, the labs continue to operate when the LIMS is unavailable or under repair or modification. Lab operations can revert back to the pre-LIMS procedures as the instrumentation and data systems are still functional. The VAX-11 LIMS has the ability to track samples through the analysis process from log in to reporting. Tests, priorities, due dates and instruments can be assigned at log in and altered at any time. Sample status reports and backlog reports are always available. Instruments and data systems can be interfaced for automatic data transfer to the LIMS, eliminating data transcription time and errors. The database of information maintains all data entries, including reruns and corrections, to provide a fully documented audit trail capability. Data can be stored in removable storage media once the test results are complete and approved. Information so stored can be restored to the LIMS for retrospective searches and reporting using DATATRIEVE, the database query language, or any of the other languages including BASIC, PASCAL and FORTRAN. These languages, as well as data-manipulation programs such as RS/1 from Bolt Beranek and Newman or SAS and SAS Graph from SAS Institute, can access the LIMS database for special data reduction and analysis tasks that might arise. The VAX-11 LIMS uses a modern approach to implementing computers in the lab and is an open and modular system that can not only operate on the VAX-11 family of computers, but can grow and be customized to meet the user's needs.

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FATE OF A FRENCH FRY. Kenneth G. Krul, Clinical Chemistry and Immunoassay Systems, Beckman Instruments, Inc.

Most people have at least heard the words "cholesterol" and "triglycerides." Many of these same people even have some idea that these terms have something to do with the health of a human being. Some are able to relate cholesterol and triglycerides to the all beef patty in their Big Mac and the deep-fry oils in the French fries. Fewer are aware of the advanced methods and instrumentation available for the analysis of serum lipid and lipoprotein analysis. Medical science has defined 5 genetically determined hyperlipoproteinemias. Each has its own characteristic predominant lipoprotein patterns, genetic determinants and secondary causes. Each results in specific pathological complications. Each also has a

specific dietary treatment—and not all are treated solely by limiting dietary fats. The means of analysis used in determining the various lipoprotein fractions, their identification and quantitation, have become more and more sophisticated in recent years. Moving from long, complicated extraction and chemical procedures to rapid enzymatic and immunochemical techniques has improved the clinician's basis for diagnosis. Using these new techniques, computer- and microprocessor-driven instrumentation have made possible the mass-processing of patient samples, the automatic flagging of abnormal patterns and the storage and retrieval of patient data.

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SEPARATION AND DETERMINATION OF TRIGLYCERIDES ACCORDING TO CHAIN LENGTH AND DEGREE OF UNSATURATION BY COMBINED LIQUID CHROMATOGRAPHY/GAS CHROMATOGRAPHY. PART II. Del E. Moore and John T. Olejko, Durkee Foods, SCM Corporation, 16651 Sprague Road, Strongsville, OH 44136.

In this paper, gas chromatography (GC) analysis employing a 6- 11 level triglyceride (TG) calibration using C-33 TG as an internal standard (IS) is discussed together with a BASIC program that enables reliable unattended operation of the GC/Autosampler for up to 3 days. The GC separation employs a 2 in. × 2 mm Pyrex glass column packed with 3% JXR on Gas Chrom "Q." Complete details are given of the calibration (500:1 concentrated range, 6 calibrants), which yield unique response factions (Rf) for all carbon numbers (CN) from 30 to 60 and all calibrated levels. An HP-5880 BASIC program is described that automatically injects 1 to 4 injections per fraction; averages; computes the percentage of RSD; and saves all reports for recalculation, if required. Other features of this program are automatic control of baseline compensation and automatic turn off of flame gases, detector, detector ignitor, and column temperature at the conclusion of sample runs. Recent work adapting the methodology to capillary columns will be discussed.

Session KK Supercritical System and Technique Wednesday p.m.

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IN SITU CHEMICAL TRANSFORMATIONS DURING SUPERCRITICAL FLUID EXTRACTION OF SEED OILS. Thomas G. Squires and Tetsuo Aida, Department of Chemistry, Iowa State University, Ames, IA 50011.

Although the use of supercritical fluid carbon dioxide to extract oils from seeds is inherently attractive, extraction efficiencies are low at 40 C. At higher temperatures (60–80 C), pressures of 10–12,000 psi are required for efficient triglyceride extraction. Besides the practical difficulties of operating at higher pressures, loss of selectivity under these conditions may require subsequent purification of the extract. In addition, many end uses require hydrolysis, transesterification or some other chemical modification of the product oil. We have designed a flow system for accomplishing these reactions in situ that should enhance the extractive process and eliminate the need for subsequent processing of the extract. An extractor/reactor that has been developed for flowing supercritical fluids through a bed of seed meal will be described. Experiments determining the solubilities of the oils and product materials under the operating conditions, the effect of reactants and physical parameters on the extraction process and initial results of the chemical transformations will be presented.

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SUPERCRITICAL CO_2 EXTRACTION OF JOJOBA. J. P. Friedrich, G. R. List and G. F. Spencer, Northern Regional Research Center, USDA, 1815 N. University Street, Peoria, IL 61604.

Jojoba wax esters are used primarily in the formulation of cosmetics. The use of petroleum solvents to extract these esters from the crushed or ground seed is not acceptable to the industry; as a result, cold pressing or expelling are the extraction methods of choice. Supercritical carbon dioxide (SC-CO_2) is an ideal solvent for jojoba and separates easily from the extracted esters. Contrary to mechanical methods that leave as much as 15% of this valuable product in the press cake, SC-CO_2 extracts the esters quantitatively and yields a light-colored oil. This oil can be bleached readily with adsorbent clays and steam refined to a nearly colorless base, with chemical and physical properties equivalent to the expelled oil. SC-CO_2 extraction also efficiently removes the residual esters from the press cake. This extraction method has additional potential for concurrent fractionation of wax esters.

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DEVELOPMENT OF INDUSTRIAL PROCESSES USING SUPERCRITICAL FLUIDS, APPLIED TO FOOD AND NATURAL PRODUCTS. Raymond J. Robey and Swaminathan Sunder, Air Products and Chemicals, Inc., P.O. Box 538, Allentown, PA 18105.

Research and development activity on supercritical fluid processing has been substantial. The food and natural product industries in particular, have shown considerable interest in separations at near-ambient temperatures with an innocuous solvent. Several firms are actively engaged in commercializing potential applications. This paper will present the supercritical fluid processing facilities of Air Products and Chemicals, Inc. by describing proposed applications from the food and natural products industries as they pass through the stages of phase equilibrium measurement, feasibility studies of the gram quantities, process development studies of kilogram quantities, process engineering/economic analyses and commercial-scale plant design.

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CRITICAL FLUID EXTRACTION OF VEGETABLE OILS. John M. Moses, Critical Fluid Systems, Inc., 25 Acorn Park, Cambridge, MA 02140.

Various vegetable oils have been extracted with a variety of supercritical fluids and liquefied gases. Preliminary designs based on laboratory and pilot-scale data indicate that not only does the process yield improved crude-oil quality over conventional hexane-extracted crude oil, but both capital and operating costs are substantially reduced.

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HIGH PRESSURE EQUIPMENT SELECTION AND CAPITAL COST CONSIDERATIONS IN THE DESIGN OF SUPERCRITICAL EXTRACTION PILOT PLANTS AND COMMERCIAL UNITS. Ronald S. Cohen, Autoclave Engineers, Inc., 2930 West 22nd St., Erie, PA 16512.

Equipment selection for supercritical extraction pilot plants and commercial units involves the careful evaluation of mechanical designs, as well as capital cost considerations. Existing proven technology in the areas of automated pressure vessels and systems are directly applicable to the commercialization of the supercritical extraction process. Alternative extractor vessel designs and various proposed supercritical extraction systems for batch and continuous operating modes will be illustrated. Process automation, including microprocessor control and on-line analytical extraction processes will be shown. A brief review of existing high pressure processes involving pressures up to 60,000 psi will be presented.

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SUPERCRITICAL FLUID FRACTIONATION OF FISH OILS—CONCENTRATION OF EICOSAPENTAENOIC ACID. Val Krukonis, Phasex Corp., #1 Mill, 85 Factory Street, Nashua, NH 03060.

Eicosapentaenoic acid (EPA) is currently being studied because of increasing evidence of its therapeutic value, specifically that in the body it is a precursor to prostaglandins. EPA is present in fish oils in varying concentrations and many laboratories are carrying out research and development directed to increasing the concentra-

tion of this active species. Supercritical fluid solvents offer the ability to separate low vapor pressure, heat-labile compounds, and several fish oils were tested for their response to supercritical fluid fractionation. Enrichment of less than 5% from a base concentration of 18% was achieved with marine triglyceride using supercritical CO_2. However, the corresponding methyl esters were upgraded to a concentration of 29%. The results of this work and related SCF fractionations will be discussed.

Session LL General Biochemistry Thursday a.m.

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ISOLATION AND CHARACTERIZATION OF POLYPRENOLS FROM SEEDS. Ravi Kothapalli, Jack W. Rip and Kenneth K. Carroll, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 5C1.

A fraction enriched in polyisoprenoid alcohols was obtained from the dry seeds of a number of crop species, using Florisil chromatography and semipreparative, reverse phase high pressure liquid chromatography (HPLC). Analytical HPLC indicated that the fraction from soybeans contained a family of polyprenols, 15–22 isoprene units in length. This material consisted entirely of α-saturated polyprenols (dolichols). Other dicotyledonous species examined (navy bean, mung bean, pea, peanut and rapeseed) also contain only dolichols. The family of alcohols resolved on HPLC from monocotyledonous species (wheat, rye, barley, rice and corn) differed from that obtained from dicotyledons as the individual homologue peaks were split and appeared to contain nearly equal amounts of 2 different components, which could not be separated by thin layer chromatography (TLC). A quantity of purified, split-peak material was obtained from wheat germ and exposed to manganese dioxide to oxidize specifically any α-unsaturated polyprenols present. The polyprenol aldehydes formed were readily separated by TLC from dolichols, which are resistant to oxidation. When the oxidation mixture was run on TLC, spots corresponding to both authentic dolichol and α-unsaturated polyprenol aldehyde were observed. The identity of the 2 components was confirmed by analytical HPLC and by IR/NMR spectroscopy. Polyisoprenoid alcohols were present in seeds at 1–16 mg/100 g with the highest concentrations in oil seeds. Some differences in homologue size and percent homologue distribution were also observed. The simultaneous presence of both α-unsaturated and α-saturated polyprenols in some species suggests that the α-unsaturated form may be the immediate precursor of dolichol.

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PARTIAL SPECIFIC VOLUME AND PREFERENTIAL HYDRATION OF LDL SUBFRACTIONS. Talwinder S. Kahlon, Gerald L. Adamson, Laura L. Glines, Joseph R. Orr and Frank T. Lindgren, Lawrence Berkeley Laboratory, Bio-Med Division, Donner Laboratory, 1-315, University of California, Berkeley, CA 94720.

We have determined the partial specific volume ($\bar{v}$) for 5 human low-density lipoprotein (LDL) subfractions (n=5–7) and evaluated preferential hydration (n=2) for LDL subfraction 3 in normolipoproteinemic subjects in order to characterize these highly atherogenic components of the human plasma lipoprotein spectra. Mean values for $\bar{v}$ were 0.9757 ± 0.0019; 0.9701 ± 0.0007; 0.9674 ± 0.0016; 0.9616 ± 0.0016, and 0.9550 ± 0.0025 mL/g for subfractions 1, 2, 3, 4, and 5, respectively. Thus, true densities (ρ) obtained from $\rho = 1/\bar{v}$ for respective LDL subfractions were 1.0249, 1.0308, 1.0337, 1.0399, and 1.0471 g/mL, respectively. The preferential hydration values were determined in D_2O-NaCl and H_2O-NaCl-NaBr solutions. Preferential hydration of lipoprotein subfraction 3 in NaCl-H_2O solution was 3.2 to 5.0 wt %, whereas values were much lower (0.3 to 0.6 wt %) in a NaCl-NaBr-H_2O solvent system. Unhydrated densities at 1 g and 200,000 × g (used in flotation velocity determinations) for LDL subfraction 3 (n=2) were 1.0274 and 1.0308 g/mL, respectively, indicating that these LDL fractions have 22% and 50% higher compressibility than the solvent at 200,000 × g force. We observed that the linearity of ηF_0 vs ρ may not be valid for solvents NaCl-NaBr-H_2O of density 1.4744 g/mL. Thus, flotation velocity data using extremely high salt concentrations (1.4744 g/mL and higher) may be viewed with caution.

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MEMBRANE CHOLESTEROL OXIDATION AND CHOLESTEROL EPOXIDE FORMATION. Alex Sevanian, Institute for Toxicology, School of Pharmacy, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90033.

Cholesterol oxidation commonly produces cholesterol 5α,6α (αCE) and 5β,6β (βCE) epoxides among several other products. In previous studies, the course of cholesterol oxidation and associated formation of cholesterol epoxides (CE) was largely examined in pure or micellar forms of the sterol as well as in lipid mixtures containing other peroxidizable lipids. In the majority of cases, CE formation was shown to correlate with total cholesterol oxidation. Attempts to identify the relative yields of αCE and βCE following oxidation invoked by several methods indicated that the relative formation of conflicting results are noted. This has often been caused by methods used to identify the epoxide diastereomers. In this study, cholesterol oxidation was examined in model membranes (liposomes) composed of 75 mol percentage bovine liver phosphatidylcholine (PC) with the remaining lipid being cholesterol. The liposomes were subjected to various peroxidizing systems previously shown to oxidize unsaturated lipids and cholesterol. Following oxidation, ^{3}H-cholesterol and ^{14}C-αCE were added in trace amounts as recovery standards. After solvent extraction, samples were applied to silica-based diol extraction columns that allowed separation of cholesterol, and its oxidation products, from PC. The cholesterol fraction was analyzed by high pressure liquid chromatography for the content of cholesterol, αCE and βCE. Lipid peroxidation was monitored by measuring thiobarbituric acid reacting products. The results showed that the extent of lipid peroxidation, cholesterol oxidation and CE formation were highly correlated. Conditions supporting propagation of lipid peroxidation yielded the highest amounts of CE per mol of cholesterol oxidized. Moreover, βCE formation correlated with the extent of peroxidation, but this was not the case of αCE. Formation of αCE appeared largely limited to initiation-type peroxidations and was not related to the duration or degree of lipid peroxidation. This was also evidenced by the nearly exclusive formation of αCE in saturated PC liposomes subjected to similar oxidizing conditions. The significance of these findings to membrane lipid peroxidation will be discussed.

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OXYGENATION OF LIPIDS AND RELATED HYDROPHOBIC COMPOUNDS BY CYTOCHROME P-450. M. J. Coon and Lee D. Gorsky, Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109-0010.

Cytochrome P-450, a highly versatile catalyst in the oxygenation of lipids and related compounds, is widespread in nature, occurring in plants, yeast, bacteria, and almost all animal tissues. The substrates include fatty acids, prostaglandins, and steriods, as well as xenobiotics such as hydrocarbons, pesticides, herbicides, and alcohols. The hydroxylation reaction is as follows, where RH represents the substrate: $RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$. In addition, P-450 is capable of generating hydrogen peroxide: $O_2 + NADPH + H^+ \rightarrow H_2O + NADP^+$. Alternatively, P-450 acts as a peroxygenase in a reaction requiring neither molecular oxygen or reducing equivalents: $RH + XOH \rightarrow ROH + XOH$, where XOOH is hydrogen peroxide or any of a variety of organic hydroperoxides or peracids. Morgan, Koop, and Coon [J. Biol. Chem. 257:1395 (1982)] have reported that, in a reconstituted enzyme system containing alcohol-induced isozyme 3a of liver microsomal P-450, the sum of acetaldehyde generated by the monooxygenation of ethanol and hydrogen peroxide produced by the NADPH oxidase activity is inadequate to account for the O_2 and NADPH consumed. Studies on the stoichiometry have revealed an additional NADPH oxidase reaction involving a 4-electron transfer that is presumed to yield water: $O_2 + 2\ HADPH + 2H^+ \rightarrow 2\ H_2O + 2\ NAPH^+$. The occurrence of a peroxidase reaction in which free H_2O_2 is reduced to water by NADPH was ruled out. When the 4-electron oxidase activity is taken into account, measurements of NADPH oxidation

and O_2 consumption are in accord with the amounts of products formed in the presence of various P-450 isoenzymes, either in the absence or presence of typical substrates, including those that undergo hydroxylation, N- or O-demethylation, or oxidation of hydroxymethyl to aldehyde groups.

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LIPIDS IN ECOLOGY. Henry W. Kircher and Susann M. Duperret, Department of Nutrition and Food Science, The University of Arizona, Tucson, AZ 85721.

Two species of *Drosophila* endemic to the Sonoran Desert are *D. nigrospiracula,* which feeds and breeds on decaying saguaro cactus (*Carnegiea gigantea*), and *D. mojavensis,* which feeds and breeds on decaying organ pipe cactus (*Stenocereus thurberi*). In the laboratory, in the absence of *D. nigrospiracula, D. mojavensis* can also use decaying saguaro, but because of competition by *D. nigrospiracula* adults, it is rarely found associated with this plant in nature. *D. nigrospiracula,* on the other hand is never found on decaying organ pipe cactus in nature and is unable to use this plant as a substrate in the laboratory. Saguaro cactus is a typical plant. It contains ca. 2% lipids, of which 1% are simple isoquinoline alkaloids and the other 1% is made up of C_{16}-C_{18} fatty acids and common phytosterols. Organ pipe cactus is much more complicated chemically. In addition to large quantities of triterpene glycosides (glucose, rhamnose plus 3 pentacyclic triterpene acids), it contains 8–14% lipids. The lipid fraction is composed of ca. 2/3 triterpene mono, di, and triols and ca. 1/3 3β,6α-sterol diols partly esterified with C_8, C_{10}, C_{12} fatty acids. As the cacti decay, the lipids are partially hydrolyzed. The various lipid and triterpene glycoside constituents of organ pipe cactus were separately added to decaying saguaro cactus and tested with *D. nigrospiracula* and *D. mojavensis.* The triterpene glycosides, unhydrolyzed lipids and pentacyclic triterpene mono, di and triols were all innocuous to both species. *D. mojavensis* larvae were much more tolerant than those of *D. nitrospiracula* to modest concentrations of free C_8, C_{10}, C_{12} fatty acids and 3β,6α-sterol diols, and the presence of these compounds in decaying organ pipe cactus is probably the main reason why this plant is not a host for *D. nigrosporacula.* The adaptation of *D. mojavensis* to the toxic action of these compounds enables it to use organ pipe cactus without competition from other species of *Drosophila.*

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FATTY ACID COMPOSITION OF RAT MILK DURING LACTATION. Joel Bitman and D. L. Wood, USDA, Beltsville, MD 20705, and Carol S. Fink, Teresa H. Liao, P. Hamosh and Margit Hamosh, Georgetown University Medical Center, Washington, DC 20007.

Milk was collected from Sprague-Dawley rats at 1, 5, 10, 17 and 20 days of lactation. After removal of the pips for 1–2 hours, the mothers were anesthetized lightly with ether, treated with 1.0 unit of oxytocin and milked using a vacuum apparatus. Milk was stored at −70 C until analyzed. Total lipids were extracted in chloroform-methanol and fatty acid composition of the milk fat determined by gas liquid chromatography of fatty acid methyl esters. The greatest changes were observed between the concentrations of fatty acids in milk from day 1 (colostrum) and day 5. Only slight changes occurred thereafter. Concentrations of medium-chain fatty acids (8:0, 10:0, 12:0 and 14:0) increased from 7.7% in colostrum to 26.4% at day 5. Compensatory decreases were observed in palmitic, oleic and linoleic acids. Long-chain polyunsaturated fatty acids also decreased as lactation progressed. Comparative data will also be presented on the variations in lipid composition at similar stages of lactation in human and bovine milk.

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CHANGES IN MEMBRANE LIPID CONSTITUENTS IN RBC AFFECTED BY RADICAL PRODUCING SYSTEMS AND THE EFFECT OF TOCOPHEROL AS A RADICAL SCAVANGER. Hiroshi Tamai, Masayuki Miki, Shigeo Nakagawa and Makoto Mino, Osaka Medical College, Department of Pediatrics, 2-7, Daigakucho, Takatsuki-City, 569, Japan.

An antioxidant mechanism of tocopherol in biomembranes was studied using red blood cells (RBC). The radical production was carried out by 2 methods: the hypoxanthine-xanthine oxidase reaction system and the autodegradation of an azo-compound producing carbon radicals. When used the first system, phosphatidylethanolamine and phosphatylserine decreased most markedly in RBC deficient in vitamin E, where arachidonic (20:4) and docosahexaenoic (22:6) were simultaneously reduced. The TBA reactive substances, conversely, increased parallel with the decrease in 20:4 and the phospholipid constituents. The degradation of membrane lipids appeared in an all or none phenomenon at the tocopherol level of around 150 μg/dL packed cells, while no change in tocopherol and the lipids occurred as tocopherol concentration in RBC membranes was more than 150 μg/dL. When the second system was used, only 20:4 decreased in RBC membranes, whereas little change occurred in the phospholipid constituents. The decrease in 20:4 in the second reaction was, however, smaller than that in the first reaction. By increasing the tocopherol concentration in RBC, the lipid changes were completely inhibited in both reactions. Contrary to the first reaction, the second reaction reduced the tocopherol concentration lineally. When tocopherol continuously decreased in the membranes by the reaction at the levels of less than 150 μg/dL, the lipid change then occurred.

Session MM Protein and General Nutrition Thursday a.m.

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PLASMA CHOLESTEROL AND LIPOPROTEINS AND LDL CLEARANCE IN MINIATURE SWINE FED FATS AND PROTEINS OF VEGETABLE AND ANIMAL ORIGIN. Lynnette S. Walsh, Donald C. Beitz and Norman L. Jacobson, 311 Kildee Hall, Iowa State University, Ames, IA 50011.

We have found that diets based on vegetable fat and protein, compared with animal fat and protein, cause increased cholesterol deposition in tissues of swine. Changes in LDL-cholesterol and/or LDL-protein, induced by dietary fat or protein source, may affect LDL uptake via receptors and cholesterol uptake via cell-surface exchange. We used LDL, labeled with [^{14}C]-sucrose (covalently bound to apolipoprotein B) and with [^{3}H]-cholesterol to determine if dietary fat and protein source alters LDL and affects tissue cholesterol deposition by these mechanisms. In a 2 × 2 factorial design, 7-week-old miniature pigs were fed, for 6 weeks, 4 diets containing vegetable fat and protein (soybean) and animal fat (beef tallow) and protein (egg white). Egg yolk was used to raise daily cholesterol intake to 30 mg/kg body weight. Plasma cholesterol, lipoprotein cholesterol and LDL protein were measured weekly. After isolation and labeling with [^{14}C]-sucrose and [^{3}H]-cholesterol, LDL were reinjected into donor pigs. Plasma radioactivity was monitored for the next 2 days. Plasma cholesterol concentrations for pigs fed soy protein and egg white were 92 and 93 mg/dL, and for those fed beef tallow and soybean oil were 96 and 89. LDL cholesterol tended to be lower, and HDL cholesterol greater, in pigs consuming beef tallow compared with pigs consuming soybean oil. Cholesterol/protein (w/w) in LDL was lower ($P<.05$) at week 6 in pigs fed beef tallow compared with pigs fed soybean oil (0.676 vs 0.781). Soy protein caused a greater decrease ($P<.02$) in LDL cholesterol/protein over time than did egg white. The half-life of both LDL protein and cholesterol were greater when pigs consumed soybean oil; soy-protein consumption resulted in a greater half-life of LDL cholesterol and a shorter half-life of LDL protein.

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TISSUE CHOLESTEROL CONCENTRATIONS OF YOUNG PIGS FED BEEF, SOY AND CONVENTIONAL DIETS. Deborah A. Diersen-Schade, Marlene J. Richard, Donald C. Beitz and Norman L. Jacobson, Iowa State University, 313 Kildee Hall, Ames, IA 50011.

Previous work in our laboratory demonstrated that young pigs fed a restricted diet containing soybean oil and soy protein isolate deposited more cholesterol in body tissues than did pigs fed a restricted diet containing ground beef. To examine the effects of more liberal feeding of beef, soy, and conventional pig diets on cholesterol deposition, 21 6-week-old male pigs fed in pairs for 7 weeks on

corn-based diets containing ground beef (B), soybean oil-soy protein isolate (S), or soybean meal (C) at 90–95% of ad libitum intake. Fat of B, S, and C diets contributed 53%, 52%, and 8% of calorie intake. The B and S diets were supplemented with dried egg yolk to increase cholesterol intake to 40 mg/kg body weight daily. Vitamins and minerals were added to meet requirements. At slaughter, B-fed pigs had greater body weights and cholesterol concentrations in blood plasma, HDL, and LDL than did C-fed pigs, with S-fed pigs being intermediate. Diet did not affect cholesterol concentration in heart, skeletal muscle, or brain. B- and S-fed pigs had more cholesterol and total lipid in liver and carcass than did C-fed pigs; in the remaining viscera and aorta, C-fed pigs had greater cholesterol concentrations. S-fed pigs had greater cholesterol concentrations in subcutaneous and perirenal adipose tissues than did B-fed pigs, with C-fed pigs being intermediate. Overall, C-fed pigs deposited substantially less cholesterol and total lipid in the body than did B- and S-fed pigs.

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SIGNIFICANCE OF SOYBEAN PROTEIN HYDROLYSATE ON CHOLESTEROL METABOLISM IN RATS. Michihiro Sugano and Akiko Yashiro, Kyushu University, Laboratory of Nutrition Chemistry, Fukuoka 812, Japan.

Soybean protein isolate (SPI) compared with casein lowers serum cholesterol levels of humans and experimental animals. As the protein effect should ultimately be attributable to the amino acid composition or sequence of that protein, we have compared the digestion process of SPI and casein in the alimentary tract of rats by aiming at possible formation of "physiologically active" fragment(s) from SPI. SPI was digested in the stomach to a number of oligopeptides with molecular weight 1,000–2,000 and macropeptides (molecular weight ca. 5,000), while no seizable oligopeptides were found with casein. Those oligopeptides with a high arginine/lysine ratio were characteristic for a SPI meal. Both SPI and casein were digested in vitro by pepsin at pH 1.6 to 2 fractions like the case of in vivo digestion. Thus, oligopeptide production was pH-dependent. When rats were fed the cholesterol-enriched, semipurified diet containing 20% SPI-pepsin hydrolysate, the serum cholesterol-lowering effect was evident. Possible participation of lower molecular weight fragments as a trigger for the protein effect is now under study. No predictable differences were found in the molecular pattern and amino-acid composition of the soluble fraction of the intestinal contents between rats fed the SPI and casein diets. These results suggest the important role of the initial step of protein digestion in the regulation of serum cholesterol levels.

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HYPERCHOLESTEROLEMIA INDUCED BY DIETARY CASEIN IN RABBITS: INVOLVEMENT OF THE AMINO-ACID COMPOSITION AND STRUCTURE OF CASEIN. C. E. West, K. E. Scholz and A. H. M. Terpstra, Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, and A. C. Beynen, Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen and Department of Laboratory Animal Science, State University, Yalelaan 1, 3508 TD Utrecht, The Netherlands.

In young, growing rabbits, cholesterol-free, semipurified diets containing casein as a protein source produce hypercholesterolemia, but no such effect is observed with soybean protein. How, in molecular terms, dietary protein affects cholesterol metabolism in the rabbit, is not known. In the present study, we have investigated whether the differential effect of casein and soybean protein on serum cholesterol levels is related to differences in the amino-acid composition and structure of the proteins. For this purpose, amino-acid mixtures simulating the intact proteins and formaldehyde-treated proteins were fed to rabbits. Formaldehyde-treatment affects the cross-linking of the protein chains. Rabbits were fed cholesterol-free, semipurified diets containing 42% (w,w) casein or 21% casein plus 1 of the following nitrogen sources: soy isolate, amino-acid mixture simulating casein, amino-acid mixture simulating soy isolate, formaldehyde-treated casein or formaldehyde-treated soy isolate. Two further groups of rabbits were fed the 42%-casein diet and the diet containing casein plus soy isolate to which 0.4% (w,w) pure formaldehyde was added, this amount being identical to the amount of formaldehyde present in the diets with formaldehyde-treated proteins. The diet containing 42% casein, to which no formaldehyde had been added, induced severe hypercholesterolemia, the level of serum cholesterol after 8 weeks being about 10 mmol/L. The hypercholesterolemia was markedly reduced (>50%) by the replacement of half of the casein by soy isolate, formaldehyde-treated soy isolate or formaldehyde-treated casein. Formaldehyde per se did not significantly influence the level of serum cholesterol. When half of the casein was replaced by an amino-acid mixture imitating either casein or soybean protein, serum cholesterol levels were somewhat lower, but no differential cholesterolemic effect from the amino acid mixtures was found. We conclude that the difference in structure of intact casein and soy isolate is more important in determining the cholesterolemic responses in rabbits to these proteins than the difference in amino-acid composition.

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NEURALLY MEDIATED COMPONENTS OF THE REPRODUCTIVE FAILURE FOLLOWING EXPOSURE TO PERSISTENT, CHLORINATED PESTICIDES. Lynda Uphouse, Department of Biology, Texas Woman's University, P.O. Box 23971, Denton, TX 76204.

Chlordecone (Kepone®) is a polycyclic, chlorinated cage compound developed for use as an insecticide. Like its sister compounds, DDT and Mirex, chlordecone was once widely used in the south and southwest. Since 1975, when an industrial accident led to chlordecone pollution of the James River, the toxicity of chlordecone has been widely publicized. Both central nervous system (CNS) (e.g., tremors) and reproductive (e.g., sterility) toxicity occur after chlordecone exposure. The contribution of neural events to the reproductive dysfunction was examined by determining the ability of chlordecone to compete in vitro for the CNS estradiol cytosol receptor, determining the effect of chlordecone in vivo on hypothalamic-pituitary function and by evaluating the effect of chlordecone on behavioral and vaginal estrus in ovariectomized female rats. Chlordecone competed with estradiol for binding to the CNS (hypothalamic-preoptic-pituitary) estradiol receptor in vitro. In vivo, the pesticide produced translocation of the receptor to the nucleus. Chlordecone mimicked estradiol in vitro by decreasing luteinizing hormone (LH) and increasing prolactin (PRL) of ovariectomized rats 36 hr after treatment. In intact females, the proestrous LH surge was abolished. Initial vaginal effects of chlordecone were characteristic of estrogenic stimulation. Vaginal cornification was present 2–3 days after treatment. However, with chlordecone, cornification persisted for 19–23 days in ovariectomized females and 7–8 days in cycling females. Behavioral parameters of hormonal facilitation failed to occur and the pesticide reduced the behavioral receptivity induced by estrogen-progesterone treatment. These results suggest that chlordecone's neuroreproductive effects involve both facilitation and antagonism of hormonally mediated events. Disruption by chlordecone of reproductive competence offers a potential hazard to the fitness of nontarget species.

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THE ROLE OF SOY PHOSPHOLIPID FRACTIONS IN BIOAVAILABILITY OF DIETARY LIPID. Tom R. Watkins, CUNY, Nutrition and Food Science, 425 East 25th Street, W709, New York, NY 10010.

Specific phospholipid classes, individually or in mixtures, exert distinct influences on the digestion and absorption of dietary lipid. In a series of feeding trials, gerbils were fed diets without phospholipid (−PL), with mixed phospholipid (+PL), or 98% phosphatidylcholine (+PC). Purified diets contained (weight %): casein, 15.0%; coconut oil, 13.0%; safflower oil, 3.0%; corn starch, 52.0%; vitamins, 0.6%; salts, 6.3%; cellulose, 6.1%; and soy PL, 4.0%. Additional coconut oil replaced PL in −PL diet. Weanling gerbils fed control (+PL) and test (−PL) diets for 12 days attained final weights of 52.7 g (19.5% gain) and 40.2 g (7.2% loss). In 4-day recovery tests, 2 parallel −PL groups were given, on day 12, the +PC or +PL diet.

The +PC group resumed growth at the same rate as the controls by day 2, with a 6.9% weight gain; the +PC group barely maintained their weight, 0.4% loss. Concomitant with growth retardation, dietary lipid accumulated in the duodenum, in mg/0.5 cm: control (+PL), 4.6; test (−PL), 62.1. In 4-day recovery, fat in +PL was reduced to 20.8, but +PC group rose to 69.2. Body weights and lipid accumulation data differed significantly ($P<0.005$), except for 4-day weights. To adapt to the malabsorption, the small bowel elongated in the absence of proper phospholipid, lengths in cm: +PL, 25.7; −PL, 34.6; after 4-day recovery diets, +PL, 29.7; +PC, 34.5. Gut diameters enlarged also, in mm: +PL, 5.02; −PL, 6.50; after 4-day recovery, +PL, 5.47; and +PC, 7.08. Significant liver enlargement occurred in the −PL group compared with +PL controls. The efficacy of mixed soy phospholipids, or a non-PC fraction, in promoting optimal gut anatomy and function, and hence lipid bioavailability and growth, suggests the importance of non-phosphatidylcholine soy phospholipid in the preabsorptive and absorptive phases of dietary fat digestion.

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NUTRITIONAL INVESTIGATIONS ON JOJOBA (*Simmondsia chinensis*) OIL. J. Décombaz and K. Anantharaman, Nestlé Products Technical Assistance Co. Ltd., Research Department, CH-1814 La Tour-de Peilz, Switzerland, and C. Heise, Department of Nutritional Science, University of California-Berkeley, Berkeley, CA.

The digestibility of fats and oils is a property relating to their chemical and physical structure. Jojoba wax (JO) is naturally a liquid consisting of esters, mainly C_{40} and C_{42}, of straight chain monoethylenic acids and alcohols. Though valued for nondietary uses, it has desirable culinary properties and no unpleasant taste. Since its nontriglyceride structure suggested low digestibility, we investigated the energy value of JO in the rat, a first evaluation of its safe use as a low-calorie fat substitute in foods. JO digestibility at 12%, 6% or 3% of an adequate, semipurified (casein) diet was ca. 40%. Diets were made isoenergetic with corn oil (CO). Appetite (energy intake related to metabolic body size) was not negatively influenced by JO when compared with CO controls. The efficiency of carcass energy deposition (retained ÷ digested) was 8% (12% JO), 18% (6% JO) and 25% (3% JO). Growth rates, poor at 12% JO, were comparable to CO controls at 6% and 3%. Acute thermic effect of single doses of JO was similar to that of CO. Ca. 5% of ingested JO was found in the carcass after feeding for 30 days. Histopathology of target organs, including the heart, revealed no untoward signs of toxicity after feeding JO at 6% for 1 or 8 weeks. The apparent digestibility of dietary N and the absorption of Ca were reduced; recovery in the urine of a single Ca overload was higher on a 6% JO than on a 6% CO diet. We concluded that JO contributes significantly lower usable energy than CO and is innocuous at the 6% level in the diet of rats. The metabolic fate of the digested fraction is, however, still unclear.

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ABSORPTION AND DISTRIBUTION OF JOJOBA OIL AFTER ORAL ADMINISTRATION TO RATS. A Bizzi and M. Cini, Istituto di Ricerche Farmacol. Mario Negri via Eritrea, 62 − 20157 Milan, Italy, and U. Bracco, Nestlé, La Tour de Peilz, Suisse.

Jojoba wax is a mixture of liquid esters with physical properties comparable to vegetable oils, but as it is an ester of long-chain fatty acids and alcohols, it does not undergo the usual enzymatic splitting as triclycerides do. In vitro lipolysis showed this property. In view of the possible use of jojoba oil as a low-calorie fat, we started nutritional studies. A single dose of jojoba wax resulted in partial absorption as indicated by jojoba recovered in intestinal wall, lymph, plasma and liver; 50% of the administered dose was found in 48 hr faecis. In vitro experiment suggested that jojoba waxes can be metabolized in liver. When jojoba oil was given in the diet (6%) for 2 months, a small amount of wax was found in several tissues. Liver, where jojoba is probably metabolized, accumulated a relatively large amount of jojoba. Rats fed jojoba grew as much as the controls but not as fast, whereas their food intake was slightly higher. If jojoba is a low-caloric nutrient, rats seem to compensate by increasing their food intake. In plasma, jojoba was carried mostly by chylomicrons and VLDL, and plasma triglycerides and phospholipids were increased by the treatment. No other effects were observed on tissue lipids. The profile of fatty acids was investigated in a number of tissues. With the exception of brain and testicles, an increase in the fatty acids present in jojoba was generally observed. This increase was at the expense of polyenic acid. These results confirm the hypothesis that jojoba is hydrolyzed in vivo. Further investigation of the oxidative capacities of mitochondria and enzymatic activity of microsomes indicate that in spite of the changed fatty acids profile, these particles are not affected by jojoba.

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PRESERVATION OF FOOD PRODUCTS WITHOUT THE USE OF PRESERVATIVES. Jon J. Kabara and Mary Beth Brady, Department of Biomechanics/COM, Michigan State University, A-439 East Fee Hall, East Lansing, MI 48824-1316.

Consumer activists continue to push for the reduction of chemical additives to foods. This situation presents both problem and opportunity for the food industry. Research efforts by food technologists have concentrated on improving the effects of food-grade additives and on finding improved functionality of existing chemicals. One approach given high priority in our laboratory is to use a systems approach to preservation. A systems approach allows us to formulate an environment hostile to microorganisms, using existing food-grade chemicals. An example of this systems approach is the incorporation of a monoglyceride, monolaurin (Lauricidin®), and lactic acid into a cheese sauce. A proprietary mixture of these 2 lipids (Lauric-Lac™) has been shown to extend the shelf life of cheese sauce and similar products from days to months. Data showing improved product stability at high (37 C), rather than low (25 C) temperature will be provided. Data about a meat emulsion system will also be given. Our results show that special attention given to choosing an emulsifier, lactic acid or other combinations of food grade chemicals, will give antimicrobial protection without the need for classic preservatives.

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STUDIES OF THE INTERACTION OF ZINC ION WITH PHYTIC ACID. W. J. Evans.

The combination of zinc ion with phytic acid was investigated under a variety of reaction conditions. With the P:Zn ratios used, i.e., 1:1 or 6:1, in the preparations, elemental analyses of the isolated products indicated P:Zn ratios of 1.0, 2.1 or 1.5 depending on the initial P:Zn ratios and the various bases and salts used in the preparations. Heats of precipitation of the interaction of zinc ion with phytic acid at initial P:Zn ratios of 6:1 were measured. The reaction is endothermic. From the value of the equilibrium constant associated with the reaction, the entropy change was calculated. The entropy change is large and positive, which is consistent with the view that when zinc ion reacts with phytic acid to form solid zinc phytate, the number of particles in the system increases.

Session NN Surfactants and Detergents III: Detergent Analysis Thursday a.m.

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THE ROLE OF INSTRUMENTATION IN DETERGENT ANALYSIS. John P. Slobogin, The PQ Corporation, Research and Development Center, P.O. Box 258, Lafayette Hill, PA 19444.

The amount of time necessary to analyze a detergent sample is usually rather lengthy when using conventional wet chemical methods; however, this time can be greatly reduced when certain instrumental methods are employed. X-Ray diffraction, plasma emission spectroscopy and ion chromatography are 3 instrumental techniques that we use routinely in our laboratory for detergent analysis. The roles that these instruments play in our analytical scheme for detergents will be discussed. For example, determining sulfate by ion chromatography rather than the conventional gravi-

metric procedure can reduce the elapsed analytical time by as much as 90%. In addition, a flow diagram of our analytical procedure for detergents will be shown and an example of an alkalinity balance to verify results will be explained.

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ANALYTICAL ^{14}N-NMR SPECTROSCOPY OF QUATERNARY AMINES. T. Michael Rothgeb and Elizabeth R. Jacobs, Procter & Gamble Company–ITC, 5299 Spring Grove Avenue, Cincinnati, OH 45217.

Nitrogen-14 nuclear magnetic resonance spectroscopy (^{14}N-NMR) is shown to be a viable technique for the rapid, nondestructive analysis of quaternary ammonium compounds. Quantitive ^{14}N-NMR analyses are performed at a Larmor frequency of 6.42 MHz on a JEOL-FX90Q. The method is shown to be applicable for the quantitation of single quaternary nitrogen compounds in complex mixtures as well as the determination of levels of mono-, di-, and trialkyl methyl ammonium chlorides in mixtures. Analyses are accomplished under conditions not involving extremes of heat or pH and are independent of solvent. ^{14}N-NMR analysis is shown to be selective for quaternary nitrogen compounds and not to suffer from interferences caused by the presence of nonquaternary nitrogen compounds. Typical analysis time is less than 1 hour for samples containing 1–5 weight percentage of quaternary nitrogen compounds. Accuracy and relative precision are +95% and ± 5%, respectively. The ^{14}N chemical shift dispersion for the quaternaries is wide enough to make it a useful technique for the identification of specific quaternaries. The chemical shifts for a number of different quaternary nitrogen compounds will be given along with several examples illustrating the identification and quantitation of quaternaries in complex surfactant mixtures.

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ANALYSIS OF QUATERNARY AMMONIUM COMPOUNDS. G. Szajer and L. Yodual, Akzo Chemie, 8401 West 47th Street, McCook, IL 60625.

The determination of the softener-antistatic agent in wash cycle fabric softeners, dryer-added softeners and detergent-softener formulations is presented. A technique using high pressure liquid chromatography identifies and quantifies the quarternary ammonium compounds. The separation is accomplished using an alumina column and a conductivity detector. The detector offers improved selectivity and detection limits over other detectors. Principal examples are separations of monoalkyl, dialkyl and trialkyl ammonium chlorides derived from tallow fatty acids. Other separations include mixtures of the above, imidazoline and ethoxylated ammonium chlorides, and sulfates. Separations of other anions such as borates and acetates are presented.

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MOLECULAR REARRANGEMENT OF FATTY ACID DIAMINES OF DIETHYLENETRIAMINES. Warner M. Linfield, Raymond G. Bistline, Jr. and Philip E. Pfeffer, Eastern Regional Research Center, USDA, 600 East Mermaid Lane, Philadelphia, PA 19118.

The reaction between 2 mol of fatty acid and 1 of diethylenetriamine yields a diamide, which cyclizes to form an imidazoline. The reaction can be followed by the American Oil Chemists' Society (AOCS) wet method procedure. This involves titration of total amine content, titration of amine content after treatment with phenylisothiocyanate and titration of amine content after treatment of a sample with salicylaldehyde. The purified diamide after treatment with salicyladehyde was not titratable with acid, indicating a primary amine structure $RCONHCH_2CH_2N(COR)CH_2CH_2NH_2$ for the diamide. However, ^{13}C NMR clearly indicated that the diamide has the symmetrical structure $(RCONHCH_2CH_2)_2NH$. When the diamide and salicylaldehyde were reacted and the products isolated quantitatively, ^{13}C NMR and IR spectrophotometry suggested the formation of a Schiff base with the following unsymmetrical structure, $RCONHCH_2CH_2N(COR)CH_2CH_2N{=}CHC_6H_4OH$. The ready formation of the Schiff base, as well as the rapid dehydration of the diamide to the imidazoline, would indicate that under conditions of heat and dehydration an unexpected migration of one of the acyl groups occurs with the subsequent loss of 1 secondary amide group and formation of a tertiary one.

Session OO Protein Mineral Interaction: Functionality and Bioavailability Thursday a.m.

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FUNCTIONALITY OF SOY PROTEIN IN MEAT INJECTION SYSTEMS. Steven Young, Archer Daniels Midland Company, 1825 North Laramie Avenue, Chicago, IL 60639.

The effect of protein-mineral interactions on soy protein functionality in brine-injected, whole-muscle meat products will be reviewed. The importance of functionality on brine performance and in the meat tissue will be stressed. Major considerations are the balancing of the proper soy proteinate (sodium or potassium), accompanying functinalities, e.g., water-binding, gelling and viscosity, the specific meat system and requirements pertaining to nutrition, processing and marketing. When properly formulated, soy protein can allow significant cost savings, increased yields and reduced fat while maintaining all quality characteristics.

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INTERACTION BETWEEN SODIUM SALTS OF CHLORIDE, PHOSPHATE AND NITRITE AND TURKEY MUSCLE PROTEINS. Kenneth J. Prusa, University of Missouri, Human Nutrition and Foods, Room 217, Gwynn Hall, Columbia, MO 65211.

In a series of studies, turkey-muscle batters were prepared with the sodium salts of nitrite, chloride and a variety of phosphates. Extracted protein amounts were measured by the biuret method and separated by SDS-gel electrophoresis and high pressure liquid chromatography (HPLC). Electrical resistance heating at 2 voltage levels was used to heat muscle batters. An Instron-monitored the expansion of the batter during heating. Generally, electrophoretically separated proteins extracted from batters with sodium nitrate had higher molecular weights than those from batters with only water. The pH of the extracts from batters with sodium nitrite were similar to those with water alone, but heated batters with sodium nitrite became less fluid (lesser peak depth) in the initial heating stages and expanded more throughout the heating cycle. When batters were prepared with sodium chloride (NaCl) alone, the following factors increased: centrifuged-batter sediment weight, concentration of total protein extracted, the number of electrophoretically separated higher molecular weight protein bands and HPLC separated protein peak areas when compared with batters prepared with water alone. Heating batters with NaCl increased expansion (Instron peak heights) and increased the temperature at which batters reached maximum Instron peak height when compared with batters without NaCl. Batters with STP (with NaCl) had increased batter expansion (greater peak heights) on heating and were the least fluid (lesser peak depths).

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RHEOLOGICAL CHARACTERIZATION OF PROTEIN CURDS FROM BLENDS OF MILK AND OILSEED PROTEINS. Y. R. Choi, E. W. Lusas and K. C. Rhee, Texas A&M University, Food Protein Research and Development Center, Faculty Mail Box 183, College Station, TX 77843.

Protein curds were prepared from blends of milk and oilseed proteins (peanut, soybean and sunflower seed) with various types of coagulants to obtain the desired texture required for making cheese analogs or developing new protein food products. Rheological properties (hardness, adhesiveness, springiness, cohesiveness and chewiness) of the curds were then determined by using the Instron Universal Testing Machine. The mechanisms involved in curd texture formation were characterized and discussed for each type of oilseed protein and coagulant before and after blending with milk

protein. The effects of anionic groups of coagulants on the texture of individual oilseed protein curds were studied. In summary, sunflower seed protein alone did not form an acceptable curd although the protein was precipitated by the coagulants used. Peanut protein formed hard, tough curd compared with soybean curd. Individual rheological properties of the curd could be controlled by properly blending oilseed and milk proteins and by choosing proper coagulants. Anionic groups of calcium and magnesium salts, which were used as coagulants, showed some degree of correlation with springiness of the curd, whereas cationic groups were correlated to curd hardness.

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BIOAVAILABILITY OF MINERALS FROM MILK-BASED AND SOY-BASED FORMULA DIETS. David A. Cook, Nutritional Science Department, Mead Johnson Nutritional Division, 2204 Pennsylvania Avenue, Evansville, IN 47721.

The dietary requirement for any nutrient is significantly related to the bioavailability of that nutrient. Scientific and regulatory personnel are placing increased emphasis on documentation of nutrient bioavailability, particularly for foods that may represent the sole source of nutrition for humans. When bioavailability data from human studies are inadequate, or cannot be obtained, results of studies with alternate models may be useful. Examples of the use and limitations of various animal models in assessing mineral bioavailability from milk-based and soy-based formulas will be presented. Results of human and animal studies documenting mineral bioavailability from these formulas will be reviewed and differences in mineral bioavailability between milk- and soy-based formulas will be considered.

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PROTEIN SOURCES AND MINERAL UTILIZATION IN HUMANS. C. E. Bodwell, Protein Nutrition Laboratory, Beltsville Human Nutrition Research Center, USDA, Room 214, Building 308, Beltsville, MD 20705.

Various nonprotein constituents found in individual or mixed protein sources have been found to have negative effects on mineral bioavailability in animal studies. These include oxalates, phytates, polyphenols and fiber, which have been shown to interfere with the use of iron, zinc or other minerals. As will be indicated by the data presented, whether or not such components have harmful effects in human diets based on either single protein sources or mixed protein sources has, for the most part, not been clearly established. The protein *per se* from soy products has been implicated in reducing iron absorption or use in humans, both when the products were consumed singly or when used to extend meat. As will be shown by data presented from studies conducted at Beltsville and elsewhere, however, these results may not be of practical importance in mixed diets. The implications of these observations will also be briefly considered in relation to non-soy plant protein sources and vegetarian diets.

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EFFECT OF PROTEIN ON IRON BIOAVAILABILITY. Dennis D. Miller and Louise A. Berner, Cornell University, 118 Stocking Hall, Department of Food Science, Ithaca, NY 14853.

The literature on iron bioavailability is replete with reports describing effects of protein (or major dietary protein sources) on iron absorption. Meats enhance, whereas plant, milk and egg protein foods depress nonheme iron absorption. Few studies designed to explain these differences have been carried out. Hypothesized mechanisms whereby proteins effect iron availability include stimulation of gastrointestinal secretions, solubilization and depolymerization of iron by amino acids or peptides and the binding of iron by undigested protein fragments. We have shown, using an in vitro system, that the amount of small molecular weight, soluble iron (dialyzable iron) released from protein-iron mixtures subjected to a simulated gastrointestinal digestion, depends on the protein. Iron dialyzability was high for bovine serum albumin and beef, intermediate for egg albumin and low for soy flour, gelatin, casein, soy-protein isolate and gluten. Low molecular weight digestion product fractions from BSA and beef enhanced iron dialysis; similar fractions from casein and soy-protein isolates had no effect. Undigested or partially digested soy protein and casein retarded iron dialysis; similar fractions from BSA and beef did not affect iron dialysis. The effects of proteins on iron bioavailability appear to be related to the affinity of undigested or partially digested protein for iron and to the stability of small molecular weight, soluble iron complexes formed from protein digestion products.

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EFFECT OF EGG PROTEIN ON IRON ABSORPTION. Josephine Miller, University of Georgia, Georgia Experiment Station, Department of Food Science, Experiment, GA 30212.

Egg yolk contains a phosphoprotein, called phosvitin, that has some unusual chemical and nutritional properties. Phosvitin makes up ca. 1% of the weight of fresh egg yolk (which contains about 16% total protein) and has 30–35 residues of serine per kg of protein. The hydroxyl group of each molecule of this amino acid is esterified to phosphates, and the resulting phosphoserine group has a high affinity for iron. Essentially all of the iron of egg yolk is bound to this protein, but the amount of iron normally present in egg yolk is not adequate to saturate its iron binding capacity. Evidence that the biological availability of egg-yolk iron is low, and that egg yolk may even interfere with absorption of iron from other sources consumed concomitantly, is attributed to binding in the gastrointestinal (GI) tract by phosvitin or its digestive products. Results of studies of iron absorption in the presence of dietary egg yolk reported in the literature are conflicting, however, and do not unanimously support the concept of interference by phosvitin. Much of the disparity is probably caused by experimental design as planning a study in which native egg iron is the only dietary variable between test and control subjects is impossible. Using anemic rats in hemoglobin repletion assays, we have compared the relative biological value (RBV) of iron in egg yolk with that of ferrous sulfate (100%). An RBV of 84% was obtained when all diets contained 14% casein, and 66% when all diets contained 20% protein, but casein was reduced in proportion to the egg protein added.

Session PP New Developments in Oilseed Products for Livestock Thursday a.m.

301

OVERVIEW OF OILSEED PRODUCTS IN THE FEED INDUSTRY. Allen Ater, Anderson, Clayton & Co., P.O. Box 2538, Houston, TX 77252.

The use of oilseed meals in the US has increased at the compound annual rate of ca. 2-1/2% since 1970. Foreign use has increased at more than double the domestic rate in the same interval. The upward trend has generally been broken only in years of short supply. The world demand outlook for oilseed products is for continued expansion, but growth patterns will vary markedly in different regions. Future US domestic use should level off in relation to animal numbers and may actually decline in some cases as more is learned about the role of specific amino acids in feed rations. The same trend may be apparent in the EEC, particularly if oilseeds and meals lose their favorable duty status. Conversely, the less-developed and central-plan countries are making serious efforts to improve feed rations by raising protein content. The long-range potential for growth in these markets is enormous, but will be tempered along the way by economic realities and political decisions. On balance, world demand for oilseed products will continue to grow but perhaps at a slower rate than in the past. Soybean meal will continue as the dominant product, but increasing emphasis will be placed on the production of other oilseeds and legumes, especially in importing countries. Because cottonseed availability will be limited by the demand for cotton, sunflower and rapeseed remain as the best choices behind soybeans to satisfy future growth in demand.

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PROTEIN UTILIZATION IN RUMINANT ANIMALS. F. N.

Owens, A. L. Goetsch and D. C. Weakley, Oklahoma State University, 208 Animal Science Building, Animal Science Department, Stillwater, OK 74078.

The postruminal supply of amino acids often limits production of ruminant animals at certain stages of life (lactation, very rapid growth of young animals). The amount of protein that escapes, or bypasses destruction in the rumen and passes to the small intestine, ranges from 0% to over 80% and varies with chemical and physical characteristics of the protein, the types and activities of microbes in the rumen and the time the protein is subjected to microbial attack. Escape of dietary protein increases when ruminal pH decreases ,when ruminal residence time is reduced and when ionophores are fed. Treating protein with heat or with certain chemicals (formaldehyde, tannins), or coating with certain polymers or lipids, can increase protein escape from the rumen. Proteins that are insoluble in acid-pepsin appear resistant to digestion in the digestive tract of the ruminant. Certain high-bypass protein sources need to be discounted for low protein digestibility and for low levels of essential amino acids. Dietary protein also supplies ammonia and possibly other growth factors (amino acids, branch-chained fatty acids) for synthesis of microbial protein within the rumen. When added dietary protein increases growth rate of ruminants, feed intake is typically increased as well, possibly through an altered hormonal status. In the future, combinations of high-escape protein sources, together with nonprotein nitrogen plus coated, enteric essential amino acids, should decrease the cost of protein supplementation or increase productivity.

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DIGESTIBILITY OF AMINO ACIDS IN OILSEED MEALS BY SWINE. D. A. Knabe and T. D. Tanksley, Jr., Texas A&M University, Animal Science Department, Kleberg Center–Room 212C, College Station, TX 77843.

Oilseed meals are the major source of supplemental amino acids in swine diets. Because these meals are expensive, the nutritionists must choose the meal wisely, to minimize cost. One factor that may enter into this decision is differences in availability of amino acids among the meals. Several methods have been used to estimate availability, the most common being the fecal index method and the ileal method. Both methods measure digestibility. Of these two, the ileal method is preferred because values are not affected by microbial action in the large intestine. Using this method, our lab has determined apparent amino digestibilities of 21 different soybean products and 6 cottonseed products. From this data, several conclusions can be drawn. Commercially processed soybean meals (n=11) have very uniform amino-acid digestibilities. Too little heat treatment of soybean products results in lower digestibilities, with Trp being the most affected. Glandless cottonseed meals have digestibilities similar to soybean meals. Glanded cottonseed meals have lower digestibilities, and, as expected, Lys is the amino acid most depressed. In all meals, Thr, Lys and Trp (when measured) were the least digestible of the essential amino acids. Work with peanut and sunflower meals is limited, but existing data indicate that these meals have digestibilities somewhat lower than soybean meals. Similarly, rapeseed and canola meal also appear to have amino-acid digestibilities lower than soybean meal.

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USE OF COTTONSEED PRODUCTS IN POULTRY DIETS. B. L. Reid, University of Arizona, Room 326 Agricultural Science Building (38), Tucson, AR 85721.

Two components limit use of cottonseed meal in poultry diets. Gossypol, a polyphenolic compound, is toxic to growing chicks in dietary amounts of 160–200 ppm and 50 ppm cause olive-green discoloration of yolks in stored eggs. During processing, a portion of the gossypol binds with the epsilon amino group of lysine, making this essential amino acid unavailable for absorption. Cyclopropenoid fatty acids, present in cottonseed oil, produce a shift to more saturated fatty acids in egg yolk and cause pink-white discoloration and yolk mottling in stored eggs. A commercially processed glandless cottonseed meal (GCSM) and an isopropyl alcohol (IPA) extracted meal were evaluated in laying-hen diets up to levels of 15%, compared with 2 regular cottonseed meals. The regular meals had free-gossypol levels of .039% and .061%; whereas the IPA meal and GCSM had levels of .002% and .012%. In the 336-day laying-hen studies, the .061% free-gossypol regular meal significantly depressed egg production and egg yields, whereas the lower gossypol regular meal and the IPA meal did not. GCSM reduced egg numbers but not egg yields. No yolk discolorations were found in eggs from birds fed GCSM. Significant increases in saturated fatty acids in yolks were obtained with the 2 regular meals but not with the IPA meal or GCSM.

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RESEARCH ON THE USE OF FULL FAT SOYBEAN PRODUCTS. Leroy J. Hanson, Triple "F" Feeds, P.O. Box 3600, Urbandale Branch, Des Moines, IA 50322.

The feeding of whole soybeans to livestock and poultry has been in commercial use in the US since the early 1970's. Various methods of cooking soybeans have been used to eliminate the inhibitors and, in some cases, rupture the oil cells. The oil in soybeans can be used as a source of energy for all livestock and poultry when formulated into balanced diets. The use of full energy soybeans is dependent on the economics and relative value of other protein and energy sources. The use of whole soybeans also incorporates the natural lecithin contained in soybean oil and other tocopherols that can act as natural antioxidants. Practical trials with poultry would indicate that the energy available from soybean oil actually exceeds the projected energy source. In swine, the added energy will normally result in an 8–12% feed efficiency advantage compared with diets without added fat. In ruminants, various processing methods can be used to decrease the degradability of the high-quality soy protein and energy to bypass the rumen into the lower intestinal tract where it can be used to increase performance, particularly in high-producing dairy cows. Low-cost processing equipment is now available that makes developing a processing technique in areas of the world where soybean processing may not be available possible. The same equipment can also be used for the processing of grain and soy combinations, which could have considerable merit for a number of other reasons.

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FATS AND OILS–DYNAMIC INTERESTING FEEDSTUFFS. Harley D. Hathaway, Buckeye Cellulose Corporation, P.O. Box 8307, Memphis, TN 38103.

Fats are the most concentrated dynamic supplemental source of calories used in feed. They contain over twice as many calories as any other feedstuff. Calories from fat are often used much more efficiently than those from carbohydrates or proteins. Monogastrics use fat well. Ruminants offer a challenge. Although fat is well digested, it can influence the rumen fermentation. The dynamic effects associated with added fat are often not obtained in ruminant diets. Advancing the technology could dramatically expand this market. Perhaps significantly, research in Ohio suggests that diets containing fiberous feeds and fat compliment each other for dairy cattle. Both fiber and fat spare grain. This advance could help feed a hungry world in the event of grain shortages. Another interesting use was developed in North Carolina. Ca. 10% added fat is used to limit the grain intake of cattle fattened on pasture. When the pasture is lush, cattle consume smaller amounts of a self-fed grain-fat mixture. As pasture decreases, cattle increase their consumption. Uniform rapid rates of gain are obtained. The total use of fats in feed can fluctuate dramatically, ranging from 500 million to 1.6 billion pounds annually in the United States. The fluctuation depends primarily on the economics of using fat in broiler feed. One percent in broiler feed is equivalent to 330 million pounds annually. Typical levels are 1–4%. When fat is too expensive, its use can be discontinued completely. The recent practice of feeding sizable quantities of whole cottonseed to cattle is of interest. Cottonseed oil is one of the most desirable oils for human food. If research provides methods of providing calories in effective, cheaper form, using by-product fats, economics will dictate against the use of whole cottonseed as cattle feed.

Meetings

Session QQ Lipid Recovery Thursday a.m.

307

INFLUENCE OF EXTRACTION METHOD ON LIPID ANALYSIS: A REVIEW. A. J. Sheppard, T. S. Rudolf and C.-S. J. Shen, Food and Drug Administration, 200 C Street, S.W., Washington, DC 20204.

Methods of extracting lipids have been of concern to chemists since the mid-nineteenth century. Henneberg and Stohmann (1885) published the basis for fat determination as used in proximate analysis. Leach (1906) published an in-depth review of the problems and achievements as related to bacteria and culture media. Bailey and Walker (1914) devised the ether Soxhlet extraction system that is currently used by AOAC. Bloor (1928) developed the "Bloor reagent" for blood plasma lipids determination. Lovern reviewed literature on lipid extraction methods as of 1942. Folch and coworkers and Bligh and Dyer worked with combinations of chloroform and alcohol to extract total lipids. More recently, the emphasis has been oriented toward the extraction of different lipid classes in addition to total lipids. The advent of more powerful separation systems has laid additional demands on extraction techniques. Sheppard (1963) demonstrated that the Soxhlet system did not extract total lipids well and did extensive damage to polyunsaturated fatty acids. Hubbard et al. (1974, 1977) reported studies indicating that the extraction method used affects the subsequent fatty acid and sterol analysis. A number of papers evaluating extraction procedures have recently appeared in the literature. A review and discussion of the interrelation between lipid extraction method and analytical results will be presented.

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EVALUATION OF SEVERAL FACTORS ASSOCIATED WITH THE DETERMINATION OF THE OIL CONTENT OF HIGH OIL CONTENT OILSEEDS BY SOLVENT EXTRACTION. James K. Daun, Agriculture Canada, Grain Research Laboratory, 1404, 303 Main Street, Winnipeg, Manitoba R3C 3G8, Canada.

The high oil content of oilseeds such as canola, flaxseed and sunflower seed present problems for analysis by extraction since oil is expressed and lost during grinding. A recent approved AOCS procedure for sunflower seed uses diatomaceous earth to scavenge this oil and allow it to be included in the analysis. For 4 types of oilseeds-canola (*Brassica napus* and *Brassica campestris*), sunflower seed, and flaxseed-the inclusion of diatomaceous earth resulted in significantly higher oil recoveries. Soxhlet extractors were more efficient than butt tube extractors, which in turn were more efficient than the Soxtec extraction apparatus in completely removing oil from the seed. Diethyl ether was found to give higher and less variable oil content estimates than hexane but the difference varied with seed type. Diatomaceous earth was found to promote oxidation of the oil when mixed with ground seed. The effect could be significant in the case of oil content determinations for flaxseed.

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PROBLEMS IN EXTRACTION AND RECOVERY OF LIPIDS FROM MARINE ORGANISMS. Jeanne D. Joseph, National Marine Fisheries Service, P.O. Box 12607, Charleston, SC 29412-0607.

In view of the current emphasis on both content and quality of lipids in human nutrition, the methodology selected for lipid extraction of seafoods must provide accurate data to permit the development of safe and prudent diets. Frequently, the modification of lipid extraction methods is required to achieve quantitative lipid recovery from marine organisms because of their diversity of form and chemical composition. Whiel quantitative recovery is of paramount importance, the safety, cost and subsequent disposal of the required organic solvents should be considered when selecting an extraction method. Another important consideration, dictating careful method selection, is related to sample preparation or processing before extraction: lyophilization and seafood processing methods such as canning, smoking or freezer storage can lead to alterations in the quantity and quality of extracted lipids. Therefore, a variety of lipid extraction methods are used in most marine lipid laboratories. Described in this paper are some of the difficulties encountered and solutions devised in the extraction of lipids from marine plants, invertebrates and finfish.

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CHANGES IN LIPID CONTENT OF CRABMEAT AS A FUNCTION OF THE PICKING PROCESS. Judith Krzynowek and Kate Wiggin, U.S. Department of Commerce, National Oceanic & Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester Laboratory, Emerson Avenue, Gloucester, MA 01930.

Crabmeat is picked from the carapace, legs and claws by many different methods. When the lipids are extracted from the crabmeat with chloroform/methanol, the amount of fatty acid methyl esters varied according to the picking method. Experiments were conducted to determine which lipid extraction method would provide clues to the mechanism for the change in lipid composition.

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VERSATILITY OF THE DRY COLUMN METHOD FOR LIPID ISOLATION. Robert A. Maxwell and William N. Marmer, USDA, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

The recently developed dry column extraction method allows lipid to be isolated from biological materials that have been blended into a matrix of Celite 545 and anhydrous sodium sulfate. By the proper choice of eluting solvents, total lipid may be isolated either as a composite extract or as individual fractions of neutral and polar lipid. This technique allows for subsequent study of polar lipids without the need for chromatographic separations. The method was originally developed for the isolation of lipid from bovine muscle and adipose tissue, and allowed the facile recovery of lipid from hundreds of tissue samples for fatty acid profiling. Without modification, the same method was found to be applicable to lipid isolation from poultry, pork, lamb, fish, and processed meat products, and to legumes. With minor modifications, the method was found to be applicable to eggs, bovine milk, and canned processed foods. Other researchers have extended the method to lipid isolation from human milk and blood. The method has also been used to isolate oxidized lipids from cooked, nitrite-treated meat. The above applications will be described and compared with traditional extraction methods.

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CHLOROFORM METHANOL (CM) AND HEXANE ISOPROPANOL (HIP) FOR EXTRACTION OF LIPIDS FROM FINNISH DIETS. Lea Hyvönen, Ulla Aulavuo and Pekka Koivistoinen, University of Helsinki, Department of Food Chemistry and Technology, Viikki, 00710 Helsinki 71, Finland, and David Homer, Valio Finnish Co-operative Dairies' Association, Research and Product Development.

The effectiveness of chloroform methanol (CM) and hexane isopropanol (HIP) (50 mL) was tested in 3-min extractions shaken by hand of prehomogenized, frozen (−20°) samples (2 g) of 1-day diets compounded by means of 24 hr recall. Prewashing was with 0.25% acetic acid, rendering both 2-phase systems and the washing of extracts unnecessary. Fatty acid methyl esters prepared by alkaline methanolysis were analyzed by the 2-column technique on 25 m OV-1 and OV-351 fused silica columns in a Micromat HRGC 412 gas chromatograph (Orion Analytica, Finland) with a Micromat HRDS data station. Forty peaks, from 6:0 to 22:6, were identified by the retention index monitoring technique. Double extraction proved to be quantitative. CM gave higher lipid values ($P<0.05$) than HIP, because of nonlipid residues. The recovery of trielaidin was 103% with HIP and 104% with CM. HIP-extracted lipid had the same fatty acid composition (mg fatty acid/100 g diet) as CM lipid. The total PUFA content found was higher than that obtained by calculations from current food composition data bases. We concluded that HIP extraction is as good as CM in dietary lipid studies presenting results as weight of each fatty acid per unit weight of diet, provided the dietary lipid consists mainly of triglyceride.