ABSTRACTS OF PAPERS

1

PRODUCTION, CHEMISTRY AND COMMERCIAL APPLICATIONS OF VARIOUS CHEMICALS FROM CASTOR OIL. FRANK C. NAUGHTON, The Baker Castor Oil Co., 40 Ave. A, Bayonne, N.J. 07002.

The presence of a hydroxyl group in addition to an olefinic linkage, in the predominating fatty acid of castor oil, gives this vegetable oil many unique and interesting properties. Castor oil consists largely of glycerides of ricinoleic acid or 12-hydroxy octadecenoic acid. Because of the high content of this unusual hydroxy acid, the oil is different in many respects from other commercial vegetable seed oils. Although a small percentage of this oil is used for medicinal purposes, it is not edible and adapts itself to a variety of chemical modifications. The chemical reactions of castor oil to derive industrial products, such as dehydrated castor oil, undecylenic acid, 12-hydroxylstearic acid, sebacic acid and nylon 11, depict the uniqueness of this agricultural oil. By dehydration, castor oil is converted to a conjugated acid oil similar to tung or oiticica oil. The catalytic dehydration results in the formation of a new double bond in the fatty acid chain. The dehydrated castor oil imparts good flexibility, rapid dry, excellent color retention and water resistance to protective coatings. The pyrolysis of castor oil cleaves the molecule to produce undecylenic acid and heptaldehyde. The pyrolysis of the methyl ester at 450–550 C results in the formation of methyl 10-undecylenate. Hydrolysis of the methyl ester gives 10-undecylenic acid. Hydrogen bromide is added to form 11-bromo undecanoic, which is ammoniated and condensed to form a nylon polymer. When castor oil is added slowly to an 80% caustic solution, the sodium ricinoleate formed splits to form sodium sebacate and capryl alcohol. By separating sodium sebacate from excess caustic and acidifying, sebacic acid is recovered. Sebacic acid is condensed with hexamethylene diamine to form nylon 6–10. The commercial application of castor oil derivatives in urethanes, starch gel modifiers, medium chain triglycerides and thixotropic additives is briefly reviewed.

2

FEEDING ENCAPSULATED OILS TO INCREASE THE POLYUNSATURATION IN MILK AND MEAT FAT. L.F. EDMONDSON and JOEL BITMAN, Dairy Products Lab., ARS, USDA, Rm. 1639 South Bldg., Washington, D.C. 20250.

The polyunsaturation in milk fat can be increased more than 10-fold by feeding dairy cows a specially prepared encapsulated or "protected" vegetable oil. Encapsulated oil may be prepared by spray drying a formaldehyde-treated, homogenized blend of the oil and a solution of sodium caseinate. The formalin reacts with the protein to form a coating around the fat globules which is stable at the pH of the rumen, thus protecting the encapsulated fats from microbial hydrogenation. Encapsulated safflower oil fed to cows in amounts of 0, 130, 260, 495, 1040 and 1480 g per day (safflower oil equivalent) during succeeding weekly intervals yielded C 18:2 levels in the milk fat of 2.1, 6.1, 9.3, 16.1, 22.1 and 30.0% of total fat, respectively. Milks containing high levels of C 18:2 developed an oxidized flavor, which was very slight in fresh raw milks, but increased markedly after 24 hr. Addition of an antioxidant (tocopherol) to the freshly drawn milk diminished the development of oxidation. Milk fat from cows fed "protected" oil showed changes in physical and chemical properties expected of a highly unsaturated fat, e.g., in fats containing 30% C 18:2, the inception melting point, determined by DTA, showed a shift from —21 C to —43C. Butter and whipping creams containing high levels of polyunsaturation required modified processing conditions. Polyunsaturation of meat fat can also be increased by feeding "protected" oils. In one experiment, 4-day-old bull calves were fed milk containing 14% C 18:2 fatty acid in the milk fat for 10 weeks, followed by an 8 week period on dry feed containing "protected" safflower oil. The C 18:2 in their depot fats was about four times that in fat from calves on "protected" rations. Blood cholesterol, triglycerides and nonesterified fatty acids all increased markedly as cows were fed increasing amounts of "protected" feed.

3

USE OF OLEIC ACID DERIVATIVES TO ACCELERATE DRYING OF THOMPSON SEEDLESS GRAPES. VINCENT PETRUCCI and NICK CANATA, California State University, Fresno, Calif., and H.R. BOLIN, A.E. STAFFORD and G. FULLER, Western Regional Research Lab., ARS, USDA, Berkeley, Calif. 94710.

The usual method of converting grapes to raisins is to harvest the grapes by hand and subsequently place them on paper trays spread between the vine rows in the field. Approximately 3 weeks of exposure to the sun allows the grapes to dry to 14–15% moisture which is characteristic of commercial raisins. Drying time for grapes can be shortened effectively if the waxy cuticle on the grape skins can be modified to allow faster movement of water from inside the grapes. One method for accomplishing this cuticle modification is to apply small quantities of oleic acid or oleic acid derivatives to the grapes before drying. Such application allows accelerated drying in the sun and in commercial dehydrators. In recent experiments at California State University at Fresno, drying of grapes prior to harvesting was accomplished. This paper will relate application of oleic acid derivatives by various means to drying rates of Thompson seedless grapes and to flavor of the product raisins.

4

USE OF HEXANE-ACETIC ACID TO PREPARE OIL AND PROTEIN FROM GLANDED COTTONSEED. T.P. HENSARLING, T.J. JACKS and L.Y. YATSU, Southern Regional Research Lab., P.O. Box 19687, New Orleans, La. 70179.

Mixtures of hexane and acetic acid (hexane containing 0–25% acetic acid v/v were used to prepare oil and protein from glanded cottonseed by solvent extraction. As the amount of acetic acid in the solvent increased, the amounts of total lipid, phospholipid, neutral oil, and gossypol in each miscella increased. The amount of free fatty acids in each miscella did not change. However the solubility of protein in 0.02 N NaOH decreased as the amount of acetic acid in the solvent used to prepare each meal increased.

5

ADSORPTION OF COLOR BODIES FROM WATER-WASHED SOYBEAN OIL. LEON LEVINE, Procter and Gamble Co., Winton Hill Technical Center, Cincinnati, Ohio 45224.

This work examines the equilibria of adsorption of color bodies on bleaching earths. The system studied was Filtrol-105 as an adsorbent for color bodies found in water-washed soybean oil. The effects of temperature and oil and earth moisture have been studied for bleaching temperatures of 200–350 F in the absence of oxygen. From these data heats of adsorption for chlorophyll and "red colors" have been inferred, from which earth savings attainable via increased bleaching temperature can be predicted. The most interesting result of this work is the demonstration of the existence of an optimal oil moisture in the vicinity of 0.1%. This optimum exists at different earth moistures. This result would indicate that conventional vacuum bleaching is not an optimal process; a preferred process will be suggested.

6

DEODORIZATION ARTIFACTS FROM LINOLENIC ACID. R.G. ACKMAN, S.N. HOOPER and J. HINGLEY, Fisheries Research Board of Canada, Halifax Lab., P.O. Box 429, Halifax, N.S.

The commercial process of steam deodorization of vegetable oils under vacuum is shown to produce geometrical isomerization of single ethylenic bonds in naturally occurring all-*cis*-9,12,15-octadecatrienoic acid. Two of the mono-*trans* isomers, with the balance of the unsaturation being the original *cis* ethylenic bonds, can be determined by open-tubular gas liquid chromatography on polyester columns as artifact peaks on each side of the peak for the original all-*cis*-9,12,15-octadecatrienoic acid, and are formed in ostensibly equal amounts. The third isomer lies under the original all-*cis* peak. The corresponding isomer formation in linoleic acid is markedly less.

7

OIL DEODORIZING SYSTEM MODIFICATION FOR (A) HEAT RECOVERY AND (B) PALM OIL STRIPPING. ARNOLD M. GAVIN and RALPH BERGER, EMI Corp., 3166 Des Plaines Ave., Des Plaines, Ill. 60018.

With continuous deodorizers of the double shell type, most of the steam normally used to preheat the feedstock in the deaerating section can be saved by a modification, the essential feature of which is the addition of a heat recovery section, located between the final deodorizing section and the cooling section, by means of which heat is transferred from the hot deodorized oil to the feedstock. Consistent with the principles of the original design, no pumping or piping of hot oil outside the deodorizer is required. Continuous deodorizers of the stripping tray type can be modified for "steam refining" or "stripping" of high FFA palm oil to reduce the FFA to 0.03% max. This is accomplished by means of additional trays in the stripping sections of the deodorizer. The capacity, sparge steam, temperature, vacuum and retention time remain the same as with normal deodorization.

8

MEASURING THE OXIDATIVE STABILITY OF PEANUTS AND PEANUT PRODUCTS WITH THE OXYGEN BOMB. BENNY R. BLANKENSHIP, CHARLES E. HOLADAY and PHILLIP C. BARNES, JR., National Peanut Research Lab., P.O. Box 637, Dawson, Ga. 31742.

The active oxygen method (AOM) of the AOCS is used extensively to evaluate the oxidative stability of fats and oils. The AOM test lacks versatility, however, in that it can be used only for a few products such as lard and vegetable oils. Tests in our laboratory have shown that results can also differ widely between laboratories, even on the same sample. Recent work with the oxygen bomb at the National Peanut Research Lab. has shown that it is both reliable and accurate for measuring the oxidative stability of peanuts and peanut products. There are four variables that affect the results of the test with the oxygen bomb including, (a) temperature, (b) pressure, (c) sample size, and (d) sample's surface area. By altering one or more of these variables almost any fatty product can be successfully tested. Results with the oxygen bomb are compared to several others tests including AOM, organoleptic measurement, iodine number, light transmittance of the oil and oil composition.

9

MUTUAL SOLUBILITY DATA OF THE OIL OF DECCAN HEMP (*Hebiscus cannabinus*) SEEDS IN AQUEOUS ORGANIC SOLVENTS. S.I. EL-HINNAWY, M.A.M. KAMMAL and A.M. EL-AASER, Faculty of Agriculture, Ain-Shams University, Shoubra-El-Khema, Cairo, Egypt.

Mutual solubilities of Deccan hemp seed oil in different organic solvents are quite an essential process in the extraction of the oil. Both *n*-hexane and petroleum ether dissolve the Deccan hemp seed oil at any concentration at room temperature. Also alcohols as ethanol and isopropanol dissolve the Deccan hemp seed oil in amounts of 28.8% and 39% at 63 C and 26 C, respectively. Both acetone and ethyl acetate dissolve the Deccan hemp seed oil at 42% and 50%, respectively, at lower temperature, i.e., the acetone at —6 C and the ethyl acetate +8 C. Both, in case of presence of humidity in oil-solvent systems, markedly affect the solvolysis of the oil. The ternary system of isopropanol-water-Deccan hemp oil proved to be the most suitable and beneficial, as the isopropanol can tolerate higher quantities of water than other solvents under investigation.

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PROPERTIES OF THE DECCAN HEMP SEED OIL. S.I. EL-HINNAWY, M.A.M. KAMAL and A.M. EL-AASER, Faculty of Agriculture, Ain-Shams University, Shoubra-El-Khema, Cairo, Egypt.

The oils of the Deccan hemp (*Hebiscus cannabinus*) seeds could be successfully extracted with organic solvents. Ether, petroleum ether, *n*-hexane, acetone and ethyl alcohol extracted from the seeds 21.99%, 20.07%, 19.9%, 14.82% and 5.7% oil, respectively. The extracted Deccan hemp seed oil showed fine characteristic properties as the color varied from 4.2–2.2 red in the Lovibond colorimeter, refractive indices 1.4713–1.4726, viscosity 71.2–81.6 cp. sap. value 190, unsap. matter 0.95–1.9%, iodine value 102.5–111.2, acid value 1.4–1.92. The gas liquid partition chromatography of the fatty acids in this oil showed that it was composed of 45–46% linoleic, 28–29% oleic, 22–23% palmitic acid and 1–2% stearic acid together with minute amounts of myristic acid. Such properties indicate that this oil could be successfully used for edible purposes.

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EFFECT OF MATURITY, HARVEST DATE AND VARIETY ON THE FREE AMINO ACID COMPOSITION OF PEANUTS. CLYDE T. YOUNG, Dept. of Food Science, Georgia Station, Experiment, Ga. 30212, and MICHAEL E. MASON, RALPH S. MATLOCK and GEORGE R. WALLER.

An improved method for the extraction of free amino acids and a flavor-related peptide with a methanol, chloroform and water mixture was described. The effect of maturity and variety on amino acid and peptide content was examined. Glutamic acid and asparagine (includes glutamine, threonine and serine) were present in highest concentration in the mature and low intermediate peanuts. Arginine was the highest in immature peanuts. This procedure has the potential of becoming a rapid and practical assay for the measurement of field maturity.

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EFFECT OF VARIETY, PLANTING LOCATION AND IRRIGATION ON THE FREE AMINO ACID COMPOSITION OF PEANUTS. CLYDE T. YOUNG, Dept. of Food Science, Georgia Station, Experiment, Ga. 30212, and GEORGE R. WALLER, RALPH S. MATLOCK and ROBERT D. MORRISON.

Asparagine, glutamine and most of a flavor-associated peptide disappeared in shelled peanuts stored 6 months at 34 F and 60% relative humidity. The effects of regional growing location (Georgia and Oklahoma), supplemental water and variety on free amino acid contents under the above storage conditions were evaluated statistically and the responses of each factor shown.

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VARIATIONS IN THE AMINO ACID CONTENT OF PEANUT FLOUR. CLYDE T. YOUNG, Dept. of Food Science, Georgia Station, Experiment, Ga. 30212, and GEORGE R. WALLER and RAY O. HAMMONS.

A hydrolyzate procedure with a precision on duplicate samples of ±2.47% requiring 15 hr for hydrolysis for best accuracy, was described. The procedure was used to examine the amino acid composition of 16 varieties of peanuts that had a range of 24–30% in protein content of the kernels. Variation of approximately two-fold in the limiting essential amino acids (lysine, methionine, isoleucine and threonine) was found. These variations permit the development of improved quality of peanut protein.

14

ELECTROPHORETIC COMPARISON OF ARACHIN ISOLATES MADE BY TWO POPULAR METHODS OF JONES AND HORN. JAQAT SINGH, Dept. of Biochemistry, Baylor College of Medicine, Houston, Tex. 77025, and JULIUS W. DIECKERT.

Arachin was prepared from a 10% sodium chloride extract of fat-free peanut meal by two methods. In one method the extract was slowly heated to 85 C; upon cooling, the flocculated material (principally enriched with conarachin) was centrifuged out and arachin (arachin-H) was precipitated from the supernatant by dilution with cold (7 C) water. In the other method, extract was saturated to 40% with ammonium sulfate and the resulting precipitate, which represents arachin (classical arachin), was recovered by centrifugation. Arachin-H and classical arachin were analyzed by conventional polyacrylamide disc gel electrophoresis. This analysis revealed that, while both isolates had seven (two major and five minor) common components, arachin-H had at least three other components not detected in classical arachin. These results clearly indicate that arachin-H and classical arachin, considered identical by Jones and Horn, indeed represent two distinct preparations of arachin.

15

TWO SOLVENT AZEOTROPIC PROCESSES FOR THE PRODUCTION OF FISH PROTEIN CONCENTRATE. THOMAS L. MEADE, Dept. of Animal Science, University of Rhode Island, Kingston, 02881.

The most widely used process for the production of fish protein concentrate is based on exhaustive extraction with isopropanol. Alternative processes involving simultaneous azeotropic dehydration and lipid extraction followed by alcohol deodorization, and alcohol dehydration and extraction followed by hydrocarbon extraction, are also in use. In the process to be described, methanol is used to dehydrate and selectively extract phospholipids, and *n*-heptane is used to complete the lipid extraction. Fish are ground and dehydrated to the desired degree with methanol. The partially dehydrated and extracted fish protein is transferred to a multistage countercurrent extraction column. Heptane vapor is introduced at the bottom of the column and, on condensing, provides the lipid extracting solvent. The quantity of vapor introduced is adjusted to provide sufficient heat to bring about the formation of the binary azeotrope of methanol and heptane, resulting in continuous removal of methanol from the column. The water content of the fish protein entering the column is critical, in that formation of a second binary azeotrope of water and heptane is utilized to achieve complete stripping of methanol. The lipid-heptane miscella are removed from the top of the column and the extracted fish protein is removed from the bottom of the column. Desolventizing is carried out at reduced pressure. The resultant fish protein, which is free of methanol, is a low bulk density product that has been evaluated by biochemical and biological methods and found to be equivalent or superior to available fish protein concentrate.

16

STANDARDS FOR IDENTITY FOR EDIBLE OILS. ROBERT W. WEIK, Div. of Food Technology, FDA, Washington, D.C. 20204.

General discussion of United States Food Standards promulgated under authority of the Federal Food, Drug and Cosmetic Act and the Codex Alimentarius food standard program. Review of U.S. procedure for accepting standards submitted to governments by the Codex Alimentarius. Current status of recommended international (Codex) standards for edible oils which have been submitted to the U.S. for acceptance as U.S. standards.

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NUTRITIONAL LABELING. NEAL H. DUNNING, Div. of Nutrition, FDA, Washington, D.C. 20204.

Increased consumer awareness and activity is a social phenomenon of the 1970's. A considerable part of this awareness has to do with the nation's food supply. Rapidly changing life-styles and technology provide both the need and the means for changing the food supply. As the complexity of food technology increases, the need for pertinent information also increases. The Bureau of Foods is attempting to meet this need by promulgating or revising regulations on the labeling of different kinds of foods. Several of these regulations are discussed, with particular emphasis on nutritional labeling.

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FOOD PACKAGING AND SAFETY PROBLEMS. PAUL HALLMAN, Office of Compliance, Consumer Product Safety Commission, Bethesda, Md. 20016.

Hazardous substances packaged in containers identifiable as food containers raise the possibility that children may mistake the product as a food and accidentally ingest it. Most of the responsibility should reside with package designers to fabricate containers for hazardous substances which do not resemble food containers. Also, food containers should not resemble containers used for hazardous substances. Mechanical hazards such as sharp edges on snack pack cans are often associated with food containers.

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FOOD REGULATIONS AND THE CONSUMER. ARTHUR F. NOVAK, Dept. of Food Science, Louisiana State University, Baton Rouge 70803.

The consumer is subjected to a great deal of diversified information on foods and nutrition. It is difficult for him to interpret much of this information. Some recommendations will be made which will enable the consumer to have a better understanding of how the government is protecting his life and health.

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NYLON 1313, A TECHNICAL FILM DESCRIBING WORK ON A NEW POLYAMIDE AT THE NORTHERN REGIONAL RESEARCH LAB. AND THE SOUTHERN RESEARCH INSTITUTE.

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BRASSYLIC ACID: CHEMICAL INTERMEDIATE FROM HIGH-ERUCIC OILS. K.D. CARLSON, Northern Regional Research Lab., ARS, USDA, 1815 N. University, Peoria, Ill., and R.B. PERKINS and E.L. HUFFMAN.

Brassylic acid, a 13-carbon dibasic acid, is a versatile chemical having properties that suit it for a number of industrial applications. These potential uses include the preparation of such polyamides as nylon 1313 and diester plasticizers, the latter being excellent low temperature plasticizers comparable to commercial sebacates. Gas liquid chromatographic analyses of products from ozonolysis of methyl erucate have allowed characterization of effects that experimental variables, such as time, solvent and temperature, have on the process. Over-ozonization is to be avoided and, contrary to previous belief, yield losses are more likely to occur in the ozonization step than during subsequent oxidation. Methyl laurate is a major byproduct derived from thermal degradation of the ozonization mixture. Oxidative ozonolysis of erucic acid in the pilot plant follows a kinetic course established in the laboratory for methyl erucate. Sampling of the reaction mixture during pilot plant production of brassylic acid and analysis of the samples by gas liquid chromatography methods indicated that neither the ozonization stage nor the oxidation stage was markedly affected by a more than 300-fold scale-up. In pilot plant production of brassylic acid, erucic acid was ozonized batchwise at 25 C using an ozone-in-oxygen stream and glacial acetic acid as solvent. The crude brassylic acid obtained by subsequent oxidation at 100 C was washed, first with water to remove residual acetic acid and then with toluene to remove monobasic acids and other byproducts. Yields of 75–80% were routine for this 95% pure brassylic acid. Further purification to polymer grade was readily achieved by percolating a warm toluene solution of the acid through a fixed bed of activated granular charcoal followed by crystallization.

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ALLYLIC PREPOLYMERS FROM BRASSYLIC AND AZELAIC ACIDS. S.P. CHANG, T.K. MIWA and W.H. TALLENT, Northern Regional Research Lab., ARS, USDA, 1815 N. University, Peoria, Ill. 61604.

Diallyl brassylate (DAB) and diallyl azelate (DAA) were prepared and converted to prepolymers for comparison with familiar analogous products from aromatic monomers and for assessment of chain length effects. Some 21–22% of DAB or DAA could be incorporated into homopolymer chains before gelation occurred due to crosslinking. To allow a margin of safety, preparative-scale reactions were stopped when polymerization was 18.5% complete. Under these conditions average isolated yields of prepolymer per reaction were 17.5%

from DAB and 15.0% from DAA, but substantially higher overall conversions could be attained by recycling the recovered monomer fractions. Prepolymers from DAB and DAA had, respectively, $\overline{M}_n$ 28,000 and 40,000, apparent $\overline{M}_w$ 716,000 and 739,000. They contained ca. 0.8 free allyl moiety per repeating unit (RU), being similar to the commercial prepolymer from diallyl *m*-phthalate but not the more widely used one from diallyl *o*-phthalate (0.53–0.56 free allyl moiety per RU). DAB prepolymer was unique among the four studied in exhibiting significant crystallinity at low temperatures as detected by differential scanning calorimetry and X-ray diffraction. Further thermal analysis showed that the aliphatic prepolymers had greater heat stability and evolved fewer calories per double bond during curing than the aromatic ones. Like their aromatic counterparts, the aliphatic prepolymers shrank slightly (<1%) during curing to give hard crosslinked products.

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ERUCAMIDE. NICHOLAS M. MOLNAR, Fine Organics, Inc., 205 Main St., Lodi, N.J. 07644.

This product is known as *cis*-13-docosenamide, or *cis*-13-docosenoic acid amide. Erucamide has, to a great extent, replaced the use of oleylamide (oleic acid amide) because of its higher melting point and higher heat resistance. These properties are desirable because of the higher operating temperatures of newer polymers. Various derivatives are discussed, such as *n*-octadecyl erucamide, which has a still higher melting point and greater thermal stability. A summary of the literature references is presented, including a review of the patents. Size of the market is also discussed. This type of product is used principally as a slip additive, an antiblock agent, and for paper-coating compositions and water-proofing. Erucamide and some of its derivatives are approved for use in polymers at low concentration levels. At these levels, the material is effective without affecting other physical characteristics of the polymer.

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USE OF FATTY ACID DERIVATIVES IN HIGH-PROTEIN BAKERY PRODUCTS. CHO C. TSEN, Kansas State University, Dept. of Grain Science and Industry, Shellenberger Hall, Manhattan 66506.

High-protein bakery foods, particularly breads and buns, are ideal foods for fighting protein malnutrition in poverty areas of the world. Fortifying wheat flour with a high level of soy flour or other protein additives can, however, induce adverse action on dough properties and bread quality. A number of fatty acid derivatives have recently been found to effectively alleviate the adverse action of protein fortification. As a result, acceptable high-protein breads and other bakery foods have been produced with the addition of sodium and calcium stearoyl-2 lactylates, ethoxylated monoglycerides, sucrose esters such as sucrose monopalmitate, sucrose mono- and distearates, and sucrose tallowate. To elucidate the improving mechanism of fatty acid derivatives, studies have been conducted to examine lipid-protein and protein-protein interactions in doughs and model systems. Results will be presented and discussed.

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PROGRESS REPORT OF THE FLAVOR NOMENCLATURE AND STANDARDS COMMITTEE. T.H. SMOUSE, Anderson Clayton Foods, 3333 N. Central Expressway, Richardson, Tex. 75080.

The Flavor Committee originated in 1967 with its scope being to standardize the nomenclature for flavors in edible fats and oils and to define these flavors in terms of the minimum number of known chemical compounds. Since the flavor of an edible food is most important in determining its commercial value, industry utilizes panels of expert tasters to grade and/or rate both the ingredients as well as the finished products. However it is important that the various panels are capable to detect and discriminate slight differences between samples. They must also rate and describe the various off-flavors detected on a uniform and consonant scale, so there is agreement among the various industries producing similar products. To determine this agreement, collaborative studies will be presented that were conducted with as many as 14 expert panels consisting of approximately 100 tasters. These panels consisted of industrial, academic and governmental groups conducting research in edible fats and oils or manufacture of fats and oils for consumer consumption. The statistical agreement between these panels in quality rating various oils will be discussed. To effectively measure the contribution of a particular chemical to flavor, carriers with little or no flavor must be utilized. A collaborative study was conducted in selecting this carrier, and data will be presented showing the blandness of certain oils. The committee has also attempted to produce oils with typical oil off-flavors by adding synthetic chemicals to bland carriers or by treating soybean oil by various processes. Data will be given that have been statistically analyzed to evaluate the agreement between the participating panels.

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CORRELATION STUDIES OF VOLATILE COMPONENTS OF VEGETABLE OILS AND PEANUT BUTTERS WITH FLAVOR SCORES. H.P. DUPUY, S.P. FORE and L.A. GOLDBLATT, Southern Regional Research Lab., ARS, USDA, P.O. Box 19687, New Orleans, La. 70179.

Flavor-scored samples of vegetable oils peanut butters were inserted directly into the liner of a gas chromatographic inlet which was carefully packed with volatile-free wool. The sample of oil or peanut butter diffused onto the glass wool, but no material was permitted to seep onto the column packing. The volatiles were rapidly eluted from the properly heated sample and concentrated on the top portion of the relatively cool column as the carrier gas was forced through the liner, and after an initial hold period of ca. 20 min., the liner with the spent sample was removed from the heated inlet. The volatiles were then resolved by temperature programing the column. On careful examination of the volatiles' profiles of the flavor-scored oils, there appeared to be a relatively good correlation of the flavor scores with their pentanal and hexanal contents. For the peanut butters, there appeared to be a very good correlation of the flavor score with the ratios of the contents of methylpropanal to hexanal and of methylbutanal to hexanal.

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CHEMICAL AND ORGANOLEPTIC PROPERTIES OF OXIDIZING OILS. J.A. FIORITI, M.J. KANUK and R.J. SIMS, General Foods Corp., Technical Center, 250 North St., White Plains, N.Y. 10625.

The oxidative stability of two animal fats (beef tallow and lard) and three vegetable oils (hydrogenated soybean, high-oleic safflower and corn) have been studied at 37.8 and 60 C. The progress of oxidation has been followed by organoleptic evaluation as well as objective quantitative tests. These include peroxide, benzidine, thiobarbituric acid, pentane and octanoic acid values; the rate of oxygen absorption has also been measured. A correlation between the quantitative tests and the objective evaluations will be discussed.

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ISOLATION AND IDENTIFICATION OF KETO FATTY ACIDS FROM MILK FAT. J.L. WEIHRAUCH, C.R. BREWINGTON and D.P. SCHWARTZ, Dairy Products Lab., ARS, USDA, Washington, D.C. 20250.

Approximately 1% of the glycerides of milk fat are comprised of keto fatty acids (KFA). Their isolation, fractionation and characterization as methyl esters was accomplished using the following sequence of steps: (a) conversion into 2,4-dinitrophenylhydrazones (DNP's), (b) adsorption of the DNP's on MgO to eliminate the colorless lipid, (c) fractionation of the DNP's into non-KFA and KFA fractions on Al_2O_3, (d) separation of the KFA DNP's into saturated and unsaturated classes by argentation column chromatography, (e) separation of these classes by chain length using liquid-liquid column and thin layer partition chromatography, (f) resolution of positional isomers by thin layer chromatography on silicic acid, (g) regeneration of the positional isomers, and (h) analysis of the parent KFA by gas liquid chromatography-mass spectrometry. In this manner 36 saturated and 12 unsaturated KFA were positively or tentatively identified. The saturated KFA ranged in chain length from C_{10}-C_{24} (predominately C_{18} and C_{16}) and generally contained an even number of C atoms, although some odd C members were also detected. The unsaturated KFA ranged from C_{14}-C_{18} with C_{18} predominating. Many saturated and polyunsaturated KFA were present which were not identified because of insufficient sample, lack of authentic standards or unusual spectra.

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FLAVOR ENHANCEMENT OF SPRAY-DRIED BUTTER. M.E. MATTHEWS, C.H. AMUNDSON and R.C. LINDSAY, University of Wisconsin, 914-B Eagle Heights, Madison, Wis. 53705.

Commercially available butter flavor concentrates and blends of pure compounds commonly implicated in butter flavor were butter. A bland water-in-oil emulsion was used as the flavor vehicle. Results, in conjunction with headspace gas liquid chromatography, indicated that flavor concentrates with a profile including dimethyl sulfide, acetaldehyde and diacetyl at taste panel evaluated with respect to the flavor of sweet cream approximate levels of 50 ppb, 0.5 ppm and 2.0 ppm, respectively, were the most effective in simulating butter flavor. Concentrates dominated by aliphatic aldehydes and ethyl esters were poorly ranked. Best ranked commercial samples and blends of pure compounds were used to prepare flavor-enhanced spray dried butter powders which ranked better than an unflavored control in taste panel evaluations. Recoveries of major flavor compounds, determined by headspace gas liquid chromatography, were 12.4% for dimethyl sulfide and 33.4% for diacetyl. Powders were produced subsequently in which volatile compound losses incurred in spray drying were compensated. A lard-based flavor-enhanced product was also prepared. These products will be evaluated as shortenings by baking laboratories.

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IDENTIFICATION OF CHICKEN FLAVOR ALDEHYDES BY PARTIAL HYDROGENATION OF THEIR DINITROPHENYLHYDRAZONES. K. DE JONG, Unilever Research, Olivier van Noortlaan 120, Vlaardingen, The Netherlands.

A flavor concentrate from cooked chicken meat was investigated by means of modern separation techniques. Several aldehydes were found, originating mainly from arachidonic acid according to the autoxidation mechanism proposed by Farmer and Sutton. The aldehydes were identified after separation by gas liquid chromatography followed by conversion into dinitrophenylhydrazones (DNPH's). In order to determine the chain length and the number of double bonds the unsaturated double bonds were partially hydrogenated and analyzed by thin layer chromatography. As catalyst, paladium on calcium carbonate in chloroform was used; as a result, only the double bonds of the aliphatic part of the DNPH's were attacked. Apart from the expected aldehydes the occurrence of 4 *cis*-decenal in cooked chicken meat was established using the partial hydrogenation technique.

30A

LIPID-SOLUBLE COMPONENTS OF MEAT FLAVOR AND ODORS. J.D. SINK, Meat Lab., Div. of Food Science and Industry, Pennsylvania State University, University Park 16802.

A review of the lipid-soluble constituents important in the flavor of various meats is presented. In addition, the focus of current research at Pennsylvania State University in these areas is discussed. Special emphasis is given to the flavor of aged beef, swine sex odor, mutton flavor and the flavor of poultry, including both chicken and turkey meat. The data presented include both chemical analyses and the results of sensory evaluation. Metabolic pathways and theoretical mechanisms in the formation of meat flavor components are presented and discussed.

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SITES OF ACTION OF STEROL BIOSYNTHESIS INHIBITORS IN *Chlorella*. G.W. PATTERSON, P.J. DOYLE, L.G. DICKSON and J.T. CHAN, Dept. of Botany, University of Maryland, College Park.

Two of the best known sterol biosynthesis inhibitors are triparanol and AY-9944. Both of these compounds have been used extensively in animals but rarely in plants. In animals, the effects of both drugs have been rather specific. Triparanol inhibits the 24(25)-reductase system and AY-9944 inhibits the 7(8)-reductase system. With both inhibitors, and in each

Chlorella species examined, the major sites of inhibition were at points other than those for which the drugs were supposedly specific. In *Chlorella emersonii* triparanol inhibited the second alkylation reaction at C-24 and the removal of the 14-methyl group. In *C. ellipsoidea* triparanol inhibited 7(8) reductase in addition to those sites seen in *C. emersonii*. In *C. sorokiniana*, which apparently does not perfrom a second alkylation at C-24, removal of the 14-methyl was inhibited, but the primary point of inhibition seemed to be at the isomerization of the 8(9) double bond to the 7(8) position. The effect of AY-9944 has been studied in *C. ellipsoidea* and *C. emersonii*. The major effect in *C. emersonii* was also an accumulation of 14-methyl sterols as with triparanol, but no accumulation of 24-methylene sterols occurred. In *C. ellipsoidea* over 90% of the sterols isolated from treated cells contained 8(9) or 8(9), 14 double bonds. These data have been combined to compare the effects of these drugs and the different biosynthetic pathways in *Chlorella* species.

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STEROLS IN *Neurospora crassa*. C.G. ELLIOTT, J.A. FREELAND and B.A. KNIGHTS, Dept. of Botany, University of Glasgow, G12 8QQ, Scotland.

The sterol contents of conidia and mycelium have been studied during the growth and development of *Neurospora crassa*. Conidia contain cholesterol as the principal sterol. Ergosterol is formed in the fungus following spore germination, and the amount of easily extractable sterol per unit weight of mycelium rises to a maximum after 3 days or then shows a decline, followed by a rise in aged cultures. The possibility of a change in the conjugated form of this sterol in the mycelium has been investigated. A number of minor sterols have been detected and partially characterized. These results will be described in detail and the role played by sterols in this organism will be discussed.

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DISTRIBUTION AND BIOSYNTHESIS OF FUNGAL STEROLS. JOHN D. WEETE, Dept. of Botany and Microbiology, Auburn University, Ala., and JOHN L. LASETER.

Sterols are widely distributed in nature and appear to play important roles in the growth and reproduction of plant and animal organisms. This also appears to be true for the fungi which are treated in this article as fungi and not plants or animals. Ergosterol is considered the fungal sterol but is not predominant in some species and may not be produced by certain lower fungi. As the more advanced methods of separation and identification are being applied to sterols of fungal origin, it is evident that these organisms are capable of producing complex mixtures of sterols such as those found in other systems. Over thirty individual sterols have been reported from various fungal organisms. The distribution of sterols and the modes of ergosterol biosynthesis as they occur in fungi are discussed.

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ALKYLATION OF C-24 IN HIGHER PLANTS, ALGAE AND LICHENS. J.R. LENTON, F.F. KNAPP, L.J. GOAD and T.W. GOODWIN, University of Liverpool, England.

It is well established that alkylation at C-24 in plant sterols involves either one or two transmethylation steps. However the mechanisms involved in various organisms are not clearly established, although they appear to be different in the alga *Ochromonas malhamensis*, the slime mould *Dictyostelium discoideum* and higher plants such as *Nicotina tabacum*. Experiments with (CD$_3$)-methionine and (2-^{14}C[4*R*]-4-^{3}H$_1$) mevalonic acid have shown that three different routes can be recognized, one in barley, one in the algal symbiont (*Trebouxia* sp. 213/3) of the lichen *Xanthoria parientia* and one in *O. malhamensis*. The evidence of these conclusions will be discussed in detail.

35

TALL OIL REFINERY WASTE WATER TREATMENT SYSTEM. LEO F. CIESIELSKI, Arizona Chemical Co., P.O. Box 2447, Panama City, Fla. 32401.

This paper describes an efficient recovery system for oils incorporated in recycle and waste water from tall oil fractionation operations. The basic system consists of API separators and a floating skimmer. Further improvements are also discussed.

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AIR AND WATER POLLUTION CONTROL IN CRUDE TALL OIL MANUFACTURE IN THE PULP AND MILL. A.B. ADAMS, The Rust Engineering Co., P.O. Box 101, Birmingham, Ala. 35201.

In recent years the pulp and paper industry has found it economically essential to recover tall oil to the maximum practical extent to help offset rising operating costs. The advent of strict air and water pollution control legislation has made it absolutely necessary to remove tall oil and other potential contaminants from the mill's wastes. Tall oil contains many fatty acids and other organic compounds that will vaporize when heated. This can occur in the evaporator section of the mill. Also, they would add a considerable BOD load and color contribution to the effluent if not removed. Pollution preventive measures should be built into the process when a new mill is designed. Corrective measures must be taken on existing mills. For air pollution control, these measures consist essentially of enclosing all vessels that contain the black liquor from which the tall oil is recovered. Hoods are placed over storage tanks, sumps, heat exchangers and other liquor-containing vessels. The hoods must be vented to a ductwork system that brings the off-gases to a central point for disposition. Typical devices to remove the offensive odors and particulate matter in the off-gases are wet scrubbers, incinerators and distillation columns. Evaporation can be used to concentrate liquids containing small amounts of contaminants to much smaller volumes and to concentrations that permit incineration. The lime kiln and recovery boiler of the typical kraft mill are commonly used to burn the odorous gases, thus destroying the odors completely. Sometimes a separate incinerator is required. Water pollution is best prevented by careful design and operation of the various tall oil removal equipment such as soap skimmers, level controls and valving systems. In spite of great care in design and operation, some tall oil will enter the wastewater stream. The effluent treatment plant must be designed to take care of this residual BOD load and, in some cases, provide for color reduction in the treated effluent.

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RECOVERY OF CRUDE TALL OIL IN A CONTINUOUS CENTRIFUGAL PROCESS. HANS BECKMAN, The De Laval Separator Co., De Laval Bldg., Poughkeepsie, N.Y. 12602, or Alfa-Laval AB, Separation Div., Fat and Oil Processing, 147 00 Tumba, Sweden.

The removal of black liquor from sulphate soap by centrifugal separation in combination with washing of the soap by adding an electrolyte is described. The electrolyte (the neutralized spent acid water from the splitting section) is mixed into the soap before it is fed to an all-hermetic separator. The soap leaves the separator as the light phase, and the mixture of black liquor and washing electrolyte as the heavy phase. Unwashed soap contains ca. 4% of thiolignin by weight. The addition of washing electrolyte and subsequent separation reduces the thiolignin content of the soap to less than 0.3%. At the same time soap concentration is increased from an average of 45% to 58–60% expressed as tall oil content. In comparison, completely decanted soap has a concentration of 63%. The main features and capabilities of a continuously working plant for splitting of sulphate soap (several of which have been in operation in northern Europe since the beginning of the 60's) are also described. The reaction between the sulphate soap and the diluted sulphuric acid or waste acid from chlorine dioxide preparation is completed in less than 5 min at a pH of 3.5–3.8, which roughly corresponds to an acid excess of 2%. The crude tall oil is separated from the spent acid water in a solids-ejecting centrifugal separator. More stringent environmental pollution regulations have necessitated the development of a system for the destruction of gases exhausted from the plant. The components of this system are described, as well as a new way of mixing alkaline solutions containing sulfide (such as white liquor) with acidic liquids to be neutralized or with lignin to be redissolved, thus preventing formation of undesirable hydrogen sulfide.

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PHYSICAL-CHEMICAL AND BIOLOGICAL TREATMENT ALTERNATIVES FOR A TALL OIL PLANT EFFLUENT. CARL E. ADAMS, JR., Associated Water & Air Resources Engineers, Inc., 2907 12th Ave. South, Nashville, Tenn. 37204.

Comprehensive treatability investigations were performed for a complex highly concentrated tall oil manufacturing plant wastestream. The studies were concerned with removing emulsified materials and treating the remaining biodegradable constituents to BOD concentrations in the range of 20–40 mg/liter. Various chemical and pH adjustment techniques were examined to remove the emulsified oils from solution. The most effective method of destroying the emulsions was found to be pH adjustment followed by coagulation to remove the free oils from solution. Various chemical coagulants were examined, including lime, calcium chloride, ferrous sulfate and ferric chloride, with the most reliable and practical chemical being ferric chloride at a pH range of 5.5 to 7.5. Several polymers were also investigated, as was coagulation effectiveness at different temperatures. After removal of the emulsified oils, the residual organics were subjected to biological treatment by facultative oxidation ponds and aerated lagoon processes. No toxicity effects were observed in either system, but the aerated lagoon process achieved more consistent, lower effluent BOD concentration. The final treatment system consists of a 1 day equalization basin, pH adjustment of the equalized waste to a level of 3.0 to break the emulsions, followed by coagulation with 400–600 mg/liter ferric chloride. Finally the coagulated stream is biologically treated by an aerated lagoon to obtain BOD levels of 25–40 mg/liter. Comprehensive treatability investigations were performed for a complex highly concentrated tall oil manufacturing plant wastestream. The studies were concerned with removing emulsified materials and treating the remaining biodegradable constituents to BOD concentrations in the range of 20–40 mg/liter. Various chemical and pH adjustment techniques were examined to remove the emulsified oils from solution. The most effective method of destroying the emulsions was found to be pH adjustment followed by coagulation to remove the free oils from solution. Various chemical coagulants were examined, including lime, calcium chloride, ferrous sulfate and ferric chloride, with the most reliable and practical chemical being ferric chloride at a pH range of 5.5 to 7.5. Several polymers were also investigated, as was coagulation effectiveness at different temperatures. After removal of the emulsified oils, the residual organics were subjected to biological treatment by facultative oxidation ponds and aerated lagoon processes. No toxicity effects were observed in either system, but the aerated lagoon process achieved more consistent, lower effluent BOD concentration. The final treatment system consists of a 1 day equalization basin, pH adjustment of the equalized waste to a level of 3.0 to break the emulsions, followed by coagulation with 400–600 mg/liter ferric chloride. Finally, the coagulated stream is biologically treated by an aerated lagoon to obtain BOD levels of 25–40 mg/liter.

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TALL OIL SOAP SKIMMING: THE STATE OF THE ART. R.W. ELLERBE, The Rust Engineering Co., P.O. Box 101, Birmingham, Ala. 35201.

This paper calls attention to the increased interest in tall oil production within the past 5 years and the simultaneous 25% increase in the market price of tall oil. It points out that tall oil has grown from a relatively unknown product to a well established commodity of considerable commercial importance, which has given kraft pulp mills a renewed interest in soap skimming. This paper has also covered the "state of the art" of soap skimming. The text reveals: (a) why mills recover soap, (b) where mills collect soap, and (c) how mills recover soap. Mills recover soap because it produces a byproduct income while at the same time giving important operational advantages. The mills collect most of the soap in the evaporator soap skimmer, but significant quantities are recovered from foam towers and liquor storage tanks. The mills recover the soap continuously in specially designed skimmers two ways: with manifold valves on foam towers and storage tanks and with specially designed foam concentrators.

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MEETING POLLUTION ABATEMENT REQUIREMENTS FOR

FATTY ACID FACILITIES. K.W. BECKER and K.C. BACZEWSKI, Blaw-Knox Chemical Plants, Inc., 1 Oliver Plaza, Pittsburgh, Pa. 15222.

Abstract not available at press time.

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RECYCLE OF TALL OIL PLANT WASTE WATER EFFLUENT. D.F. BRESS, Foster Wheeler Corp., 110 S. Orange Ave., Livingston, N.J. 07039, and Sakuo Ohuchi and Akira Katayama.

This paper describes the system presently being engineered to purify aqueous effluent from tall oil distillation plant and recycle it back to the process.

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INDUSTRIAL USES FOR ANIMAL FATTY OILS. CHRISTOPHER HERMANN and J.J. MCGLADE, Mayco Oil & Chemical Co., P.O. Box 5, Bristol, Pa. 19007.

This paper reviews the sources and types of raw materials and some of the more commonly known methods of manufacturing quality animal lard oils. These oils are used in a variety of applications including the petroleum and pharmaceutical industries. Due to lard oil's affinity for metal surfaces, the petroleum industry uses lard oils as a friction modifier and as a metal wetting agent. In the pharmaceutical industry, however, lard oil is used as a defoamer and a nutrient in certain fermentation processes. Animal lard oils can further be treated with a variety of chemicals such as sulfur and chlorine to make extreme pressure additives. These products are extensively used in the manufacture of heavy duty lubricating oil formulations to include metalworking oils, gear oils and way lubricants. Products and byproducts manufactured from the sperm whale can no longer be imported into the U.S., and in some applications lard oil is replacing sperm oil.

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LUBRICANTS AND LUBRICANT ADDITIVES: I. PERFORMANCE CHARACTERISTICS OF N-MONO AND N,N-DISUBSTITUTED AMIDES AND MODIFIED AMIDES. F.C. MAGNE, R.R. MOD, and G. SUMRELL, Southern Regional Research Lab., ARS, USDA, 1100 Robert E. Lee Blvd., P.O. Box 19687, New Orleans, La. 70179, and W.E. PARKER and R.E. KOOS.

N-Mono- and N,N-disubstituted amides prepared from substituted and unsubstituted fatty acids, principally stearic and oleic acids, were evaluated as base lubricants and lubricant additives. The N-mono and N,N-disubstituted amides of oleic acid were comparable to 100 sec paraffin oil, and dioctyl sebacate in extreme pressure and antiwear performance and had with few exceptions viscosity index values in excess of 100. They appeared to hold some promise as base lubricants at normal temperatures, but failed as lubricants at subnormal temperatures (—40 C), with the possible exception of N,N-dibutyloleamide. Those incorporating the thiirane group (the epithioamides) were found to possess extreme pressure lubricant characteristics and to be noncorrosive to copper at temperatures up to 60 C and only slightly tarnishing at 100 C. In addition these epithioamides almost invariably function as extreme pressure and antiwear additives for paraffinic or diester base oils, sometimes in the dual role for both base oils. The intensiveness of these properties has been found to correlate directly with the degree of thiirane substitution in the compound. Performance in both these capacities at the same degree of epithioation has also been found to be highly dependent upon the N- or N,N-substituent groups present.

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APPROACHES TO THE SYNTHESIS OF WAX ESTERS FOR USE AS POSSIBLE SPERM OIL REPLACEMENTS. T. PERLSTEIN, A. EISNER and I. SCHMELTZ, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

In efforts to prepare wax esters chemically similar to those comprising sperm oil, selected fats or blends thereof were reduced to alcohols which were then used to esterify acids usually obtained from the same source. In a typical procedure, lard oil was reduced to "lard oil alcohol" in the presence of sodium and methyl isobutyl carbinol in xylene. The resulting sodium alcoholates were decomposed with urea, and the liberated fatty alcohols were used to esterify a mixture of fatty acids obtained by hydrolysis of the same fat, lard oil. In a similar way, series of wax esters were also prepared from a blend of lard, coconut and crambe oils, from fractionated tallow and from commercial grade oleic acid. Occasionally, during the course of the sodium reduction of triglycerides to fatty alcohols, a byproduct produced in low yield (5–15%) was identified as wax esters. Studies were therefore undertaken to investigate the direct formation of wax esters during the reduction of triglycerides. If successful, such a process would avoid separate saponification and esterification steps. A modified procedure was developed in which fatty sodium alcoholates, obtained during the reduction procedure, are partially decomposed with urea (to about the 90–98% level). Subsequent addition of triglyceride to this mixture results in the formation of desired wax ester in relatively short times (1–3 hr) in ca. 80–90% yields.

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POSSIBLE SPERM OIL REPLACEMENTS DERIVED FROM TALLOW, LARD AND OTHER ANIMAL FATS: PROPERTIES AND APPLICATIONS. H.E. KENNEY, A. EISNER, T. PERLSTEIN, E.T. DONAHUE and I. SCHMELTZ, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

Wax esters, prepared from animal fats and winterized when necessary, were evaluated as possible sperm oil replacements. Their relevant properties, i.e., iodine value, pour point, cloud point, average chain length, were compared to those of natural sperm oil and commercially available replacements. The wax esters were sulfurized to various levels of sulfur by methods analogous to those used industrially. The sulfurized oils were then evaluated with respect to per cent sulfur incorporated, copper strip activity, solubility in bright stock, viscosity, and antiwear and extreme pressure additive activity. They were also compared to commercially available sulfurized substitutes and to sperm oil sulfurized in the laboratory. Sulfurization parameters, i.e., reaction time, per cent sulfur added and temperature, were studied to determine their effects on the antiwear and extreme pressure characteristics of the resulting sulfurized esters, and to establish sulfurization optima. Several of the esters were sulfated and then evaluated as emulsifying agents for use in the fat-liquoring of leather. Leathers processed with such sulfated esters were compared to leathers processed with sulfated natural sperm oil and commercially available substitutes. In general, wax esters derived from animal fats showed potential utility as sperm oil substitutes in several applications.

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THE EFFECT OF SOME SUGARS AS PROOXIDANTS FOR VEGETABLE OILS. S.I. EL-HINNAWY, M.A.M. KAMAL and RAGA'A OSMAN, Faculty of Agriculture, Ain-Shams University, Shoubra-El-Khema, Cairo, Egypt.

The sugars, fructose, dextrose, maltose, lactose and sucrose showed rather interesting effects on the autoxidation of vegetable oils. It has been found that these sugars differ in their effects on the autoxidation of vegetable oils. While fructose, dextrose, sucrose and lactose showed generally a prooxidant effect, maltose was either of no effect or acted as a mild antioxidant. The magnitude of the effect of each of these sugars was also different on the various vegetable oils; consequently the rating of the effects of these sugars as prooxidants was not quite similar for all oils.

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THE EFFECT OF SOME AMINO ACIDS AS ANTIOXIDANTS FOR VEGETABLE OILS. S.I. EL-HINNAWY, M.A.M. KAMAL and RAGA'A OSMAN, Faculty of Agriculture, Ain-Shams University, Shoubra-El-Khema, Cairo, Egypt.

Some amino acids, which are natural constituents of food material, proved to have antioxidant effects, but the available information about their potential antioxidative capacity is rather limited. Amino acids cystine, cysteine, glycine, as well as the tripeptide glutathione had shown to possess, had generally high potential antioxidative capacity towards the autoxidation of vegetable oils, but differed markedly among themselves in their efficiencies as antioxidants. Cysteine was the most effective amino acid, while both clycine and cystine were the least effective; glutamic acid was of generally moderate antioxidative effect. On the other hand, glutathione was nearly closer to cysteine in its pronounced antioxidative capacity. An interesting observation here is the marked differences in the capacity of inhibiting the autoxidation of vegetable oils, found between cystine and cysteine, with the latter being considerably more effective in inhibiting autoxidation. The potential antioxidative capacity shown by the amino acids seems not dependent on the presence of primary antioxidant. Consequently their antioxidant effect is not a synergistic characteristic and it acts as chain terminators, thus breaking the oxidative chain reaction.

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THE EFFECT OF DEEP-FAT FRYING ON SUNFLOWER OILS. W.H. MORRISON, III, J.A. ROBERTSON and D. BURDICK, Richard B. Russell Agricultural Research Center, ARS, USDA, P.O. Box 5677, Athens, Ga. 30604.

Studies were made to evaluate the useful life of various sunflower seed oils for deep-fat frying. Hydrogenated and unhydrogenated sunflower oils and a commercial shortening obtained from a fast-food establishment were used to deep-fry 8 lb of raw potatoes daily for 6, 8 hr days. Samples of oil were taken daily and AOM values determined. A plot of the log of the AOM values vs. time gave a straight line, the slope of which reflects the oxidizability of the oil. The partially hydrogenated northern sunflower oil was much less prone to oxidation after use than the commercial shortening and had a longer useful life even with its lower initial AOM value.

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DETERMINATION OF PENTANE FORMED DURING AUTOXIDATION OF OILS CONTAINED IN SOLID SAMPLES. GIOVANNI BIGALLI, Hershey Foods Corp., 354 Yorktowne Rd., Hershey, Pa. 17033.

The determination of pentane as index of the degree of oxidation of oils has been proposed several times. The techniques presently employed involve the direct injection of oil in a gas liquid chromatographic column. The pentane is separated in the column, but part of the sample might eventually contaminate the liquid phase, decreasing its efficiency. A more difficult problem is found with solid samples in which no direct injection is possible. To overcome these limitations, a new method was devised, and a simple piece of glassware was designed to selectively strip out the pentane from the original material in one distillation-extraction step ending with the pentane dissolved in heptane, which can then be injected directly into the gas liquid chromatographic column without causing the secondary effect in the column. The results are reproducible. The quantitation is adequate for practical purposes. Experimental samples containing ground peanuts were prepared as "models" and stored under nonideal conditions. The deterioration was followed by means of pentane determination and sensory evaluation, with good agreement being found. Other samples, including rancid nuts, oils and fats, were tested. The test is also usable in liquid samples. Details of this new procedure and discussion of the results will be presented.

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INFLUENCE OF VARIOUS ADDITIVES ON PREVENTION OF PEROXIDATION OF FATTY ACIDS IN PEANUT BUTTER. ALLEN J. ST. ANGELO and ROBERT L. ORY, Southern Regional Research Lab., ARS, USDA, P.O. Box 19687, New Orleans, La. 70179.

An earlier investigation of both enzymic and nonenzymic minor constituents in peanut butter as possible sources of fatty acid peroxidation (rancidity) showed that certain metal-containing enzymes and salts containing iron and copper were the primary catalysts (J. APREA 4:186 [1972]). Lipoxygenase, the enzyme considered to be a major cause of lipid oxidation, is completely deactivated during the roasting process. In order to extend shelf-life and retain the high quality of peanut butter, methods to prevent or decrease catalytic effects of these metalloproteins and salts were conducted, employing additives accept-

able under the new FDA Food Packaging Laws. This report will present a more detailed comparison of the effects of different levels of selected metal chelating agents, oil, salts and water on peroxidation of unsaturated fatty acids in peanut butter. The practical aspects of applying these findings to commercial manufacturing processes will also be discussed.

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Dimorphotheca sinuata LIPOXYGENASE: FORMATION OF 13-L-HYDROPEROXY-*cis*-9-trans-11-OCTADECADIENOIC ACID FROM LINOLEIC ACID. H.W. GARDNER, D.D. CHRISTIANSON and R. KLEIMAN, Northern Regional Research Lab., ARS, USDA, 1815 N. University St., Peoria, Ill. 61604.

Lipoxygenase (EC 1.13.1.13) from the seed of *Dimorphotheca sinuata* oxidized linoleic acid to predominantly 13-L-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid. When the reaction proceeded at pH 6.9, the 13-hydroperoxide was the only isomer detected; but at pH 5.1, the 13-isomer was 92% of the total, the remaining 8% being the 9-hydroperoxide. At both pH's small amounts of hydroxyoctadecadienoic acid accumulated during the reaction. The minor product from the pH 6.9 reaction was analyzed as 13-hydroxy-*cis,trans*-octadecadienoic acid. The postulate advanced by many workers that dimorphecolic acid (9-D-hydroxy-*trans*-10,*trans*-12-octadecadienoic acid) is biosynthesized via a lipoxygenase product was not proved. Although the product specificity of *D. sinuata* lipoxygenase is like that of lipoxygenase type 1 from soybeans, its inactivity at pH 9 demonstrated that it is a novel enzyme.

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ADDITION OF LINOLEIC ACID HYDROPEROXIDE TO CYSTEINE. H.W. GARDNER, R. KLEIMAN and G.E. INGLETT, Northern Regional Research Lab., ARS, USDA, 1815 N. University St., Peoria, Ill. 61604.

Linoleic acid hydroperoxide reacted with cysteine catalyzed by 10^{-5}M Fe^{III} to form addition compounds of the two reactants, as well as oxooctadecadienoic and hydroxyoctadecadienoic acids derived from the hydroperoxide. The addition occurred under both aerobic and anaerobic conditions contrary to a previous study that showed α-tocopherol added to linoleic acid hydroperoxide only under anaerobic conditions. Five different ninhydrin-positive, hydrophobic products were separated by chromatography. When examined by mass, IR, and NMR, one of the chromatographic fractions was demonstrated to be an addition product.

52A

ANTIOXIDANT ACTIVITY OF ASCORBYL PALMITATE, TOCOPHEROLS AND ASCORBIC ACID. WINIFRED M. CORT, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

In the quest to use antioxidant compounds appearing in nature, extensive studies have been made on vegetable oils, animal fats, apocarotenal and vitamin A as substrates with ascorbyl palmitate, tocopherols and ascorbic acid as antioxidants. Ascorbyl palmitate at a level of 0.01% provides a useful increase in the shelf-life of vegetable oils. Alone it is better than BHT and BHA and in combinations with other known antioxidants improves the shelf-life of all vegetable oils as well as potato chips. Solubility problems with ascorbyl palmitate and other esters of ascorbic acid will be discussed. The tocopherols are more effective in protection of animal fats, carotenoids and vitamin A. A review of experiments utilizing tocopherols and tocopherol combinations will be presented. Activity of ascorbic acid, an excellent scavenger of oxygen, will be reviewed. Evidence will also be presented indicating singlet oxygen is not involved in the direct oxidation of fats and oils.

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THE INTERRELATIONSHIP BETWEEN BIOSYNTHETIC CONTROL AND THE ROLE OF STEROLS IN MEMBRANES. WILLIAM R. NES, Dept. of Biological Sciences, Drexel University, Philadelphia, Pa. 19104.

Evidence will be reviewed which indicates (a) that free sterols act as architectural components of membranes; (b) that this is their primary role; (c) that certain structural features are of major importance; and (d) that biosynthetic control is closely related to this membraneous function. Emphasis will be given to the problems that remain unsolved and the manner in which the author is searching for solutions.

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THE FUNCTION OF STERYL AND TRITERPENE ESTERS IN PLANTS. HAROLD J. NICHOLAS and AHMED ATALLAH, Institute of Medical Education and Research and Dept. of Biochemistry, St. Louis University School of Medicine, St. Louis, Mo. 63104.

A detailed study of the liquid crystalline properties of many steryl and methylated steryl esters has indicated that the position of the nuclear double bond, as well as side chain constituents in these esters, markedly influences whether or not an individual ester will be cholesteric or smectic, both, or neither. Many of the compounds investigated have been found to be intermediates in the biosynthesis of plant (and animal) sterols, and we have designed experiments to determine whether mesophase formation, as expressed by changes in viscosity with temperature and other factors, is a mere coincidence or has some specific function in the sterol biosynthetic process. Preliminary evidence indicates that steryl esters have a special role in penetrating and disrupting membranes, and that their function may lie somewhere specifically in this area, particularly during the sterol biosynthetic process.

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SYNTHESIS AND *Drosophila* FEEDING STUDIES WITH STEROLS IN THE ERGOSTANE AND STIGMASTANE SERIES. HENRY W. KIRCHER and FUMIKO V. ROSENSTEIN, Dept. of Agricultural Biochemistry, University of Arizona, Tucson 85721.

Two series of 3β-hydroxy sterols were prepared and purified in macro quantities. Ergosterol and brassicasterol were used to prepare ergostane derivatives having Δ^0, Δ^5, Δ^7, $\Delta^{5,7}$, $\Delta^{5,22}$, $\Delta^{7,22}$ and $\Delta^{5,7,22}$ unsaturation by selective hydrogenation techniques. Sitosterol and stigmasterol were converted to their respective 7-dehydro compounds, and the remaining stigmastane derivatives were obtained from these four by similar selective hydrogenation methods. The compounds were purified by column chromatography and fractional crystallization to chromatographically homogeneous, sharp melting samples. The sterols were added to a sterol deficient medium and tested in axenic culture with species of *Drosophila* and rated on their ability to promote growth, maturation and reproduction in these insects.

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BIOCHEMISTRY OF OTHER PLANT STEROIDS (SAPONINS, GLYCOALKALOIDS, PREGNANE DERIVATIVES, CARDIAC GLYCOSIDES AND SEX HORMONES). ERICH HEFTMANN, Western Regional Research Lab., ARS, USDA, Berkeley, Calif. 94710.

The biosynthesis, metabolism and possible functions of steroids other than sterols in plants are discussed.

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FREE RADICAL THEORY OF AGING: EFFECT OF DIETARY FAT ON DISCRIMINATION LEARNING. DENHAM HARMAN, SHELTON HENDRICKS and DENNIS EDDY, University of Nebraska College of Medicine, 42nd and Dewey Ave., Omaha, Neb. 68105.

Free radical reactions have been implicated in the pathogenesis of degenerative changes in biological systems. From this point of view, variations in the onset of senility may in part reflect differences in the long term ingestion of dietary components that might be expected to significantly alter the level of more or less random endogenous free radical reactions. Thus, increasing the amount and/or degree of unsaturation of dietary fat might be expected to result in an increased rate of loss of intellectual function. To evaluate this possibility the discrimination learning ability of male rats (born to mothers receiving a semisynthetic diet containing as the sole source of lipid, lard 5 wt%, lard 20 wt%, safflower oil 20 wt%, or safflower oil 20 wt% + vitamin E [20 mg/100 g of final diet] and kept on the same diet after weaning) was determined at age 6 months. In a second study some of the mothers of the above lard 20 wt% and safflower 20 wt% groups were subjected to the same test at age 12 months; these rats were started on the semisynthetic diets just after weaning. The results are tabulated below:

Total Number of Correct Responses by the Four Rats in Each Group (Trials 1 Week Apart)

Diet	1	2	3
1. Lard 5 wt%	1163	1634	1580
Lard 20 wt%	1435	1853	1400
Safflower oil 20 wt% + vitamin E	1257	2075	2820
Safflower oil 20 wt%	358	473	315
2. Lard 20 wt%	662	759	1601
Safflower oil 20 wt%	65	581	647

These data show that safflower oil has an adverse effect on intellectual function and that this effect is probably due to in vivo peroxidation of polyunsaturates present in the oil.

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AMNIOTIC FLUID LIPIDS IN NORMAL HUMAN PREGNANCY. ERIC J. SINGH and FREDERICK P. ZUSPAN, University of Chicago, Dept. of Obstetrics and Gynecology, 5841 S. Maryland Ave., Chicago, Ill. 60637.

Analysis of the constituents of amniotic fluid has become an important technique in assessing the status of the fetus; therefore the lipid analysis of human amniotic fluid was necessary. Lipids were extracted and separated by thin layer chromatography (TLC). The purity of the samples was also checked by TLC using various solvents. The content of hydrocarbons, cholesteryl esters, cholesterol, triglycerides, diglycerides, monoglycerides, free fatty acids and phospholipids were, 14.3%, 15.0%, 11.3%, 9.8%, 6.7%, 1.6%, 15.8% and 25.5%, respectively. The phospholipids were separated into phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid, lysophosphatidyl choline, diphosphatidyl glycerol and spingomyelin by two dimensional TLC in conjunction with media containing chloroform-methanol-6N ammonium hydroxide. The methyl esters derived from transesterification of the glycerides and hydrolysis of the phospholipids and the free acids directly were analyzed by gas chromatography, employing polar and nonpolar packings. The results will be presented. The cholesteryl ester and triglyceride fraction had high content of palmitoleic acid—41.6% and 26.4%, respectively. In the free fatty acids the predominant acid was palmitic followed by myristic, stearic, oleic and palmitoleic. Monoglyceride had a high content of stearic acid as compared to other components of the lipids. The most prominent acids of phospholipids were 16:0, 18:1, 18:0 and 20:4. Temperature-programed gas chromatography showed the presence of C_{15} to C_{30} hydrocarbons, and the hydrocarbons comprised three series with 47 peaks.

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PHOSPHOLIPIDS OF HUMAN ENDOMETRIUM AND MYOMETRIUM. ERIC J. SINGH and JOSEPH R. SWARTWOUT, University of Chicago, Dept. of Obstetrics and Gynecology, 5841 S. Maryland Ave., Chicago, Ill. 60637.

Lipids were extracted from human endometrium and myometrium and the phospholipids were isolated by thin layer chromatography (TLC). Two dimensional TLC was employed for the separation of phospholipids. The concentration of each fraction was determined and the fatty acid composition was identified by gas liquid chromatography of the methyl esters, using polar and nonpolar packings. A comparative study was made of the distribution of phosphatides in the tissues. The endometrium contained phosphatidyl choline, 42.0%; phosphatidyl ethanolamine, 23.0%; phosphatidyl serine, 16.0%; phosphatidyl inositol, 6.0%; phosphatidic acid, 1.0%; lysophosphatidyl choline, 2.0%; diphosphatidyl glycerol, 2.0%; and sphingomyelin, 8.0%, while the myometrium included phosphatidyl choline, 41.2%; phosphatidyl ethanolamine, 20.5%; phosphatidyl inositol, 8.5%; phosphatidyl serine, 12.3%; sphingomyelin, 8.8%; diphosphatidyl glycerol, 4.9%; lysophosphatidyl choline, 2.7%; and phosphatidic acid, 1.1%. The predominant acid in lecithin was palmitic, followed by oleic, archidonic, stearic and linoleic. The content of palmitoleic acid

was high in the lysolecithin fraction. The phosphatidyl ethanolamine, serine and inositol contained large amounts of arachidonic acid. The endometrium and myometrium phosphatidyl ethanolamine contained ca. 66% and 57% unsaturated fatty acids, respectively. It is concluded that the phospholipid composition of the tissues is characteristic for that class and they contained large amount of polyunsaturated fatty acids.

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TUMOR LIPIDS: GLYCERIDE BIOSYNTHESIS IN MINIMAL HEPATOMAS. REX D. WIEGAND and RANDALL WOOD, Depts. of Medicine and Biochemistry, Div. of Gastroenterology, N408, University of Missouri School of Medicine, Columbia 65201.

Hepatoma cells (HTC) derived from a Morris minimal deviation hepatoma (7288C) were grown on media containing 25% serum to the confluent stage at which time serum-free media containing 1-^{14}C-palmitate was added and incubation continued for 6, 12, and 24 hr. The distribution of radioactivity among the major neutral lipid and phosphoglyceride and the various fatty acids of each class was determined for both HTC cells and culture media. After 24 hr, more than 95% of administered radioactivity was recovered in neutral glycerides and phosphoglycerides, indicating that only a small amount of the fatty acid was oxidized. More than 80% of the incorporated radioactivity was found in triglycerides, phosphatidyl choline and phosphatidyl ethanolamine. Incorporation of the label into cellular triglycerides and phosphatidyl choline plateaued at 12 hr, whereas incorporation of radioactivity into phosphatidyl ethanolamine was still increasing at 24 hr; the percentage of radioactivity incorporated into phosphatidyl ethanolamine between the 12th and 24th hr nearly doubled. In contrast, the relative distribution of the label among the lipid classes in the culture media remained constant during the 24 hr. Examination of the 24 hr cellular and media triglycerides and phospholipids showed that palmitic acid represented only ca. 10% and 30%, respectively, of the radioactivity in these fractions. Most of the radioactivity was found in 18:0, 18:1 and C_{20} acids, indicating the ability of these cells to elongate fatty acids and to desaturate saturated acids to the corresponding monoenoic fatty acids. The occurrence of radioactivity in the media lipid fatty acids shorter than palmitic acid also indicates that these cells are capable of oxidation and de novo fatty acid synthesis. Labeled glyceryl ether diesters in the cells or culture media were not detected, which is in agreement with analytical data on mass determination. The fate of palmitic acid in HTC cells exposed to the labeled substrate for much shorter periods of time is currently being investigated and will be reported.

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TUMOR LIPIDS: EFFECTS OF SERUM LIPID LEVELS ON A MINIMAL DEVIATION HEPATOMA GROWN IN TISSUE CULTURE. RANDALL WOOD and REBECCA ZAUN, Depts. of Medicine and Biochemistry, Div. of Gastroenterology, N408, University of Missouri School of Medicine, Columbia 65201.

Cultured hepatoma (HTC) cells derived from a Morris minimal deviation hepatoma (7288C) were grown in gram quantities on media containing 25, 15, 10 and 5% serum. Lipids were extracted from harvested HTC cells and the growth media after which the percentage total lipid, percentage neutral lipid, percentage phospholipid, lipid class composition and lipid class fatty acid composition were determined and compared. Similar determinations were made on rat liver and a solid minimal deviation hepatoma (7288CTC) which was derived from HTC cells transplanted into a host animal. These studies were initiated to determine: (a) the minimum percentage of serum required in the media for optimum growth of HTC cells; (b) the influence of serum lipids on HTC cell lipid composition and lipid biosynthesis; and (c) the lipid requirement of HTC cells. The total quantity and neutral lipid and phospholipid percentages remained relatively constant in HTC cells, irrespective of media serum levels. Media neutral lipids and phospholipids, which differed qualitatively and quantitatively from HTC cell lipids, did not fall below the levels originally present in the culture medium, but actually increased in most cases. These data suggest that HTC cells, unlike many neoplastic cells that utilize exogenous lipids, synthesize de novo a major proportion of their cellular fatty acids and complex lipids. The net accumulation of lipids in the media further suggests that HTC cells excrete lipids. Thus far, we have been unable to demonstrate a lipid requirement for these hepatoma cells. Glyceryl ether diesters were not detected in the HTC cell neutral lipids whereas the solid hepatoma (7288CTC) contained a small percentage of this ether-linked lipid.

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ALDEHYDOGENIC LIPIDS IN HUMAN SERUM: COMPARISONS OF AGE, SEX AND HEART DISEASE. WILLIAM J. FERRELL and PIMPUTKAR MOHINI, University of Detroit, Dept. of Chemistry, Detroit, Mich. 48221.

Human serum samples from patients with known heart disease and from patients with no history or record of heart disease (normals) were collected and assayed for free and bound fatty aldehydes. The data from the normal patients was analyzed with respect to the age and sex. The aldehydogenic lipid content of the serum from the heart patients and normal patients was also compared. No correlation could be found between the aldehydogenic lipids and either age or sex. On the other hand, the heart patients showed a significant increase in free fatty aldehydes when expressed as either μmol/1.5 ml serum or as a per cent of the total aldehydogenic lipids. The bound aldehydes showed a slight decrease in the heart patients; however the values were not significant. Based on the per cent free fatty aldehyde content of human serum it was possible to predict heart disease in 85% of the samples assayed.

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METABOLISM OF PALMITALDEHYDE IN HUMAN HEART. WILLIAM J. FERRELL and KUO-CHING YAO, University of Detroit, Dept. of Chemistry, Detroit, Mich. 48221.

Palmitaldehyde, radio-labeled in different positions with either ^{3}H, ^{14}C or both, was incubated with homogenates of human heart. The results showed that palmitaldehyde was first converted to fatty acid and to only a small extent reduced to fatty alcohols. Chain shortening and elongating processes were also observed. The potential role of palmitaldehyde in the biosynthesis of alkyl ethers and plasmalogens in human heart has been tested. The results suggest that aldehydes are reduced to alcohols prior to incorporation into 0-alkyl glycerol ethers, but that incorporation into plasmalogens occurred following oxidation to fatty acids. A tentative metabolic scheme will be discussed.

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INCORPORATION OF SELECTED ISOTOPES INTO LIPIDS OF HUMANS WITH CEREBRAL LIPIDOSIS: D-GLUCOSAMINE-1-^{14}C. R.M. BURTON, S. HANDA, R.E. HOWARD, THERESA VIETTI and A. RAGAB, Dept. of Pharmacology, WUMS, 660 S. Euclid, St. Louis, Mo. 63110.

Children in their terminal phase of cerebral lipidosis, i.e., gangliosidosis (Tay-Sachs' disease) and sphingomyelinosis (Niemann-Pick's disease), were hospitalized and given radioisotope-labeled precursors. A typical study: A child with gangliosidosis was given D-glucosamine-1-^{14}C intravenously. Urinary excretion of radioactive carbon decreased by 100-fold after 2 days and 1000-fold after 10 days. Even though blood serum radioactivity was very low after 2 days, the incorporation of radioactivity into red blood cell glycolipids increased continuously for 10–15 days. Six months after glucosamine administration, post mortem analysis showed markedly elevated levels of brain gangliosides containing radioactivity. About 47% of the brain tissue gangliosides was the typical Tay-Sachs' ganglioside, i.e., galNac-(neuNac) gal-glu-ceramide. Similar studies on sphingomyelinosis will be presented and compared to the gangliosidosis study. The results of these studies on lipidosis will be contrasted to data obtained from a nonlipidosis patient, i.e., acute lymphoblastic leukemia.

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METABOLISM OF 1-^{14}C DIPALMITOYL PHOSPHATIDYL CHOLINE IN THE DEVELOPING BRAIN. GOVIND A. DHOPESHWARKAR and CAROLE SUBRAMANIAN, Lab. of Nuclear Medicine and Radiation Biology, 900 Veteran Ave., Los Angeles, Calif. 90024.

Previous work from this laboratory showed that brain phosphatidyl choline was the most radioactive component following administration of 1-^{14}C palmitic acid to adult rats. These data aroused our interest in the fate of brain PC during a period of rapid growth and lipid accumulation in the brain. Using 1-^{14}C dipalmitoyl phosphatidyl choline (^{14}C-PC) as the ip injected tracer, we sought information on uptake of the intact molecule and distribution of radioactivity between the 1 and 2 positions of PC and also into other polar lipids. ^{14}C-PC was injected ip into 13-day-old rats. Half of the total number were sacrificed 24 hr after the tracer dose, and the remainder were allowed to survive for a period of 17 days. Examination of the brain and liver lipids 24 hr after the dose showed that the PC fraction was the most radioactive component followed by EPG. Even after 17 days, the specific activity of brain lecithin was higher than that of other polar lipid fractions. Further analysis of PC isolated from brain and liver tissue using phospholipase A from cobra venom showed that the ratio of FA/LysoPC radioactivity in the brain was ca. 16 times higher than that in the liver. The data indicated that positional specificity of phospholipase activity in the brain and the liver is not the same. Judging by the data obtained from surviving animals, the enzyme specificity also seemed to be influenced by age during rapid growth and lipid synthesis in the brain.

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DEPRESSING ACTION OF DIETARY HYDROGENATED FAT ON RAT ADRENAL CORTICOSTEROIDOGENESIS. PETER O. EGWIM and FRED A. KUMMEROW, 205 Burnsides Research Lab., University of Illinois, Urbana 61801.

We have previously shown that both the concentration and fatty acid pattern of rat adrenal cholesteryl esters are uniquely modified by the feeding of a diet (HF) containing 20% partially hydrogenated soybean fat. In an attempt to find out how these modifications might influence the cholesteryl ester related functional activities of the adrenals, we have now measured the relative abilities of adrenal homogenates from rats fed two different fat diets, HF and milk fat diet (MF) for 4 and 8 weeks, to synthesize corticosteroids from endogenous cholesteryl esters in vitro. For comparison, rats fed Purina Chow (PC) were also studied. Our data show that the linoleate-adequate diets (PC and MF) led to significantly higher levels of corticosteroid output than the linoleate-poor diet (HF). The selectivity pattern in the hydrolysis of the different adrenal cholesteryl esters was also investigated, and some major differences between the dietary groups were noted. It is thought that these differences may, at least in part, be responsible for the depressing action of the HF diet on adrenal corticosteroids.

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PROPERTIES OF THE LIPASE FROM THE MICROORGANISM, *Geotrichum candidum*. ROBERT G. JENSEN, Dept. of Nutritional Sciences, University of Connecticut, Storrs 06268.

The yeast-like mold, *Geotrichum candidum*, secretes an extracellular lipase which was found to be highly specific for oleic and linoleic acids. Both are hydrolyzed from triacylglycerols regardless of position, and with substrates containing oleic acid produce diacylglycerols suitable for stereospecific analysis. Elaidic, linoelaidic and stearolic acids, and positional isomers of 18:1 other than *cis*-9 are digested at low rates as compared to the favored substrates. The enzyme does not differentiate between oleate and linoleate nor between *cis*, *trans* and *trans*, *cis*-9,12-18:2s. Small quantities of saturated acids are also hydrolyzed. The enzyme has an optimum pH of ca. 8.6 and temperature of 37 C.

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COMPARATIVE CHARACTERISTICS AND HYDROLYTIC PATTERNS OF MILK, PANCREATIC AND MICROBIAL LIPASES. KHEM M. SHAHANI, Dept. of Food Science and Technology, University of Nebraska, Lincoln 68503.

Lipases not only play an important role in fat metabolism but also bear significance in the food industry. On the one hand they produce undesirable rancid flavors in food products, but on the other hand they are essential for the development of desired and characteristic flavors in cheeses and other foods. Comparative characterization studies conducted with milk,

bovine pancreatic and microbial lipases revealed that these lipases differ greatly in regard to their physicochemical characteristics and specificity. Milk and bovine pancreatic lipase were isolated in a pure form using fractional and selective precipitation with ammonium sulfate and acetone and Sephadex chromatography. Lipases of *Achromobacter lipolyticum, Aspergillus niger, Geotrichum candidum* and *Penicillium roqueforti* were partially purified and concentrated by ammonium sulfate precipitation and subsequent dialysis. Bovine milk and pancreatic lipases possessed a pH optimum of 9 and a temperature optimum of 37 C. Both revealed similar and typical protein absorption spectra. Both appear to be —SH group containing enzymes, and were stimulated by NaCl and inhibited by mercury and iron salts. Electrophoretic and other characteristics suggest that the two enzymes are very similar. The pH optima of the microbial lipases, however, varied between 7.0 and 9.0. With triolein as the substrate, Achromobacter lipase, with an apparent pH optimum of 9.0, showed only 46% and 76% of the apparent optimum hydrolysis at pH 7.0 and 8.0, respectively. At pH 7.0 only 20% oleic acid is neutralized as revealed by the titration curves of oleic acid. Consequently its actual pH optimum might be 7.0. Milk fat was lipolyzed by the various lipases, and the gas chromatographic analyses indicated that microbial lipases hydrolyzed considerably higher levels of the long chain fatty acids and much lower levels of butyric and short chain acids. The hydrolytic profiles of milk and pancreatic lipases showed little or no selectivity. On the other hand, kid and calf pregastric, used in the manufacture of Italian cheeses, released higher levels of butyric acid and short chain acids.

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INDUSTRIAL APPLICATION OF MICROBIAL LIPASES: A REVIEW. E.W. SEITZ, International Flavors & Fragrances, 1515 Highway 36, Union Beach, N.J. 07735.

Microbial lipases are of importance in the production of a variety of food products. This has been documented by a number of investigators who have found that both exo- and endocellular lipases, i.e., glycerol ester hydrolases, are produced in situ, along with other microbial products, during processing and/or curing of foods. Such enzymes are believed vital to flavor development as well as causing textural and structural changes in foods such as mold-ripened cheese, hard cheese, dry or semidry sausage, fermented vegetables, soy sauce paste (Miso), etc. Microorganisms that are important for in situ lipase production in manufacture of cheese include *Propionibacterium shermanii* and *Penicillium roqueforti*. Most lipase enzyme preparations for application to the food industry, especially dairy products, have come from animal sources, and the use of microbial lipases has been quite limited. However use of the latter has been made feasible through the availability of commercial quantities of such enzymes from Japan and the U.S. Moreover microbial lipases and other microbial enzymes are becoming more important because of their unique properties, availability and more favorable prices. A number of reports in the literature describe the application of microbial lipases in other industrial areas as well, including the pharmaceutical industry where the enzymes play a role primarily as digestive aids, the organic chemical industry for use in solubilizing lipids and in glycerine production, the leather industry for degreasing of hides and in pollution control projects to assist in solubilization of fats in industrial and domestic wastes.

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SOME PROPERTIES OF AN EXOCELLULAR LIPASE FROM *Rhizopus arrhizus*. GILBERT BENZONANA, c/o E.H. Fischer, Dept. of Biochemistry, University of Washington, Seattle 98195.

Rhizopus arrhizus, a mold of the mucor family, excretes a very active lipase when cultivated in a medium containing corn flour and casein hydrolyzates. Pure enzyme (lipase I) isolated from a fresh preparation of *Rhizopus* has a molecular weight of 43,000 and a high carbohydrate content. Upon storage of an aqueous solution at 4 C, lipase I slowly converted to a more cationic form (lipase II) of lower molecular weight (32,000) and a glycopeptide of molecular weight 8500 is liberated. This conversion is probably of enzymic origin, for it is inhibited by DFP. Since lipases I and II do not differ greatly in specific activity (9000 as opposed to 8100, respectively) the glycopeptide does not seem to play a significant role in enzyme activity. *Rhizopus* lipase (I as well II forms), like most of the microbial lipases, in the pH range 7.5 to 9.0 and in the presence of bovine serum albumin, shows a high specificity (more than 98%) for the external chains of triglycerides. The enzyme first forms 1,2-diglycerides which then are converted to 2-monoglycerides. Glycerol appears only after 50% of the total ester chains have been hydrolyzed, and after isomerization of 2-monoglycerides into 1-monoglycerides. Like pancreatic lipase, *Rhizopus* lipase acts on micelles of short chain as well as on emulsified particles; however the activity patterns are not identical. Bile acids are potent competitive inhibitors of *Rhizopus* lipase; in contrast, with pancreatic lipase, bile acids are inhibitors of the noncompetitive type (G. Benzonana and B. Chorvath, unpublished experiments). In both cases the inhibitor, which has nothing in common with the substrate except the physical state, probably binds to the enzyme in a nonspecific way.

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STAPHYLOCOCCAL LIPASES. D.V. VADEHRA, Cornell University, Ithaca, N.Y.

A lipase-rich fraction was isolated from the cell-free supernatant of 24 hr broth culture of *Staphylococcus aureus* B-120, grown in trypticase soy broth at 37 C. Lipase from cell-free supernatant was precipitated with equal volumes of absolute ethanol. This fraction was purified further by differential precipitation at pH 8.6 and 4.3. Subsequent purification using Sephadex G-200 and Biogel 300 yielded a preparation with 350 to 450-fold increase in specific activity. The purified lipase had an optimum pH of 8.5 at 37 C. The electrophoretic mobility was -7.78×10^{-5} cm²/volt/sec. The sedimentation coefficient for the two peaks was 2.85 and 8.5, respectively, and the molecular weight was 100,000. The purified lipase hydrolyzed a variety of natural oils and fats. The amount of free fatty acids liberated from hydrogenated soybean oil (iodine value <3) was one-third, compared to natural oils and fats. Gas chromatographic analysis of hydrolyzed synthetic triglyceride, with palmitic, stearic and oleic acid at the α, β and α^1 positions, respectively, indicated that the enzyme was capable of hydrolyzing the glycerol-fatty acid bonds at all three positions. The yield was 40% palmitic, 20% stearic and 39% oleic acids. Formaldehyde, mercaptoethanol, cysteine, glutathione and terramycin had inhibitory effects on lipase activity, while hydrogen peroxide, streptomycin and sodium taurocholate had a stimulatory effect on the activity.

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ECONOMIC SITUATION AND OUTLOOK FOR TALLOW AND PALM OIL IN THE UNITED STATES. GEORGE W. KROMER, Rm. 114, Economic and Statistical Analysis Div., Economic Research Service, USDA, 500-12th St., S.W. Washington, D.C. 20250.

The fats and oils that can serve as raw materials for the manufacture of surfactants include tallow, grease, lard, palm oil, corn oil, cottonseed oil and soybean oil. But for economic reasons tallow and palm oil are the most feasible. Tallow and greases in the U.S. are utilized exclusively in nonfood products, whereas lard and the oils go mainly into food products. Most fats and oils entering the edible market command a price premium over the inedibles. Tallow output has more than doubled during the past two decades, reflecting the uptrend in livestock slaughter and meat production. Domestic markets have been unable to absorb expanding supplies and exports have increased. Exports now account for about one-half of domestic production. The principal domestic markets are animal feeds, fatty acids, soap and many other industrial applications. U.S. supplies of palm oil (all imported) have quadrupled over the past 5 years. It enters duty free, mainly from Malaysia and Indonesia. Increasing imports at competitive prices have cut into traditional markets for cottonseed and soybean oils. The potential for expanded use of palm oil in both food and industrial products looms large. World palm oil production approximately doubled during the past 5 years, with about one-half the increase in Malaysia, the leading producer. Sharp future gains are also projected for Malaysia, which exports over 90% of its production.

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ADVANCES IN SOAP TECHNOLOGY. ERIC JUNGERMANN, Armour-Dial, Inc., Greyhound Tower, Phoenix, Ariz. 85077.

Toilet soap bars have remained one of the most stable product categories from the consumer point of view. Bar soaps have resisted the inroads of synthetic detergents much more successfully than other cleaning products, and at present almost 80% of all toilet soaps manufactured are based on the traditional tallow-coco soap base. In spite of apparent technological stability there have been significant changes in production processes and the formulations of bar soaps. New continuous processes are gaining worldwide importance, while new additives are being used in formulations. The presentation will review the latest changes in these two areas and provide an update on the state of the art of bar soap technology.

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SOAP-BASED DETERGENT FORMULATIONS: V. AMPHOTERIC LIME SOAP DISPERSING AGENTS. N. PARRIS, J.K. WEIL and W.M. LINFIELD, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

A series of amphoteric surfactants was prepared by the reaction of primary fatty amines, N-methyl fatty amines and N,N-dimethyl fatty amines with either 1 mol or 2 mol of propane sultone. The uncatalyzed reaction with one equivalent of propane sultone proceeded readily to give nearly quantitative yields of products which were easily purified by crystallization. In order to bring about the reaction of primary amines with 2 mol of propane sultone, 2 mol of sodium methoxide had to be added to the reaction mixture, and the yields were poorer than in the case of the 1 mol adducts. Furthermore the disulfopropylated amines were more difficult to purify. The monosulfopropylated primary amines were less water soluble than either the disulfopropylated compounds or the analogous monosulfopropylated N-methyl or N,N-dimethyl alkylamines. Thus the Krafft point of N-(3-sulfopropyl) hexadecylamine was 58 C, that of N-methyl-N-(3-sulfopropyl) hexadecylamine, 17 C, and that of N,N-dimethyl-N-(3-sulfopropyl) hexadecylamine, 27 C. The analogous disulfopropylated fatty amine gave a clear 1% solution at 0 C. All of the mono- and disulfopropylated fatty amine derivatives were excellent lime soap dispersing agents with a lime soap dispersion requirement of 3–4, according to the method of Borghetty and Bergman. None of the surfactants possessed outstanding detergency characteristics by themselves, but in combination with tallow soap and silicate builder they exhibited a marked improvement in detergency. The quaternary amphoterics obtained from N,N-dimethyl alkylamines and 1 mol of propane sultone, when formulated with soap and builder, gave rise to the best detergency especially when the alkyl chain length was in the tallow range (C_{16}-C_{18}). These detergents when tested in 300 ppm hard water compared favorably with commercial phosphate-built detergents.

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RECENT DEVELOPMENTS IN CATIONIC SURFACTANT APPLICATIONS. H.A. GREEN, B. LIKE, A. PETROCCI and R. SORRENTINO, Millmaster Onyx Corp., 190 Warren St., Jersey City, N.J. 07302.

A number of bis-quaternary compounds have been prepared for evaluations of germicidal effectiveness, with particular emphasis on efficacy in hard water and suitability for disinfectant application. Structure-activity relationships regarding carbon number and chemical variations in bridging were developed through hard water tolerance and use-dilution tests. Correlation anomalies are noted. Results of investigations of skin degerming with combinations of tertiary amine oxides with quaternary ammonium compounds are described. Microbiological data pertinent to surgical scrub use are given, and compared to a commercial preparation based on hexachlorophene. The hair-conditioning properties of C_{12} through C_{20} tertiary amine oxides are described, and the esthetic advantages of simple formulations discussed.

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LACTOSE-DERIVED SURFACTANTS. F. SCHOLNICK, M.K. SUCHARSKI and W.M. LINFIELD, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

Lactose, the principal solid component of whey, is a

potentially inexpensive and abundantly available raw material. Its chemical structure is well suited to serve as the hydrophilic portion of a nonionic surfactant molecule. Accordingly, a series of esters of lactose was prepared by reaction of fatty acid chlorides with anhydrous lactose in N-methylpyrrolidone at 90–100 C. Crude monoesters of lauric, palmitic, oleic, stearic and tallow fatty acids were obtained in this manner. Other methods of synthesis of the products have been investigated but have been unsuccessful. These have included transesterification of fatty esters with lactose, catalyzed with potassium carbonate, as well as direct esterification of the sugar with fatty acids. The above lactose esters were evaluated for detergency behavior, emulsification time and lime soap dispersing power. The results indicate that these surfactant properties are comparable to those exhibited by the analogous sucrose derivatives. Thin layer chromatography (TLC) proved to be suitable for the qualitative evaluation of the purity of the individual esters. According to TLC, lactose palmitate was the purest of the series of esters prepared. The degree of purity of the esters clearly affected water solubility and surface active properties. The palmitate thus was the most water soluble of the series and gave the highest surfactant performance.

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ADVANCES IN POLYOL SURFACTANTS. FREDERIC R. BENSON, ICI America Inc., Wilmington, Del. 19899.

Recent developments in the chemistry and applications of lipid-based polyol surfactants are reviewed. Polyol surfactants include the partial fatty acid esters of glycerol, pentaerythritol, polyglycerols, pentitols, hexitols, anhydrohexitols, sugars and their polyoxyethylene derivatives. Besides studies on these nonionic surfactants, recent investigations include studies of ionic derivatives such as the hexitol esters of α-sulfo fatty acids and succinyl monoglycerides. Uses of polyol surfactants in foods, pharmaceuticals, agricultural chemicals and polymers have expanded. Besides serving as emulsifiers and solubilizers, they assist in extractions, function as lubricants and plasticizers, assist in the control of oil spills, and provide water-proof barriers. Factors relating applications of polyol surfactants to their composition are discussed.

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Abstracts not available at press time.

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Abstracts not available at press time.

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Abstracts not available at press time.

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Abstracts not available at press time.

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SKIN LIPIDS OF THE FLORIDA INDIGO SNAKE. DAVID G. AHERN and DONALD T. DOWNING, Dept. of Dermatology, Boston University School of Medicine, 80 E. Concord St., Boston, Mass. 02118.

Cast skins of the Florida Indigo snake (*Drymarchon corais*) yielded up to 8% of chloroform-methanol-extractable lipid, which was found to contain methyl ketones (20%), free cholesterol (15%), free fatty acids (5%), free primary alcohols (30%), free secondary alcohols (15%) and hydrocarbons (5%). The methyl ketones were predominantly straight chain compounds of 31 and 33 carbon atoms, and were mainly monounsaturated, with double bonds almost exclusively in the position 7 carbon atoms removed from the hydrocarbon end of the chain. The structures of the secondary alcohols corresponded with the methyl ketones in regard to chain length distribution, location of the oxygen function in the 2 position, and the proportion and position of unsaturation. The primary alcohols were also predominantly straight, odd carbon, unsaturated compounds, with the double bonds located 7 carbons from the hydrocarbon end of the chain, but with chain lengths principally of 29 and 31 carbons. The free fatty acids were unremarkable in structure in that they were mainly even carbon monounsaturated compounds of 16–20 carbon atoms with double bonds almost exclusively in the 9 position from the carboxyl group. The hydrocarbons appeared to be contaminants, in that gas chromatography revealed a distribution characteristic of petroleum hydrocarbons. However skin lipids from two other species of snakes housed in the same quarters contained only cholesterol, free fatty acids, triglycerides and hydrocarbons, and none of the unusual constituents obtained from several specimens of the Florida Indigo snake. Inspection of the lipid structures obtained from the Indigo snake suggests a biogenetic relationship whereby palmitic and palmitoleic acids are extended in chain length to mainly 32 and 34 carbon-atom fatty acids retaining a keto group in the 3 position. Decarboxylation could then yield structures corresponding with the methyl ketones and, by reduction, the secondary alcohols. Baeyer-Villiger-type insertion of an oxygen atom between carbons 2 and 3 of the methyl ketones followed by hydrolysis of the acetates thus produced would yield the series of odd carbon primary alcohols observed. Similar series of interrelated compounds have previously been identified only in bacteria, where the proposed biogenesis has been established, but the methyl ketones are known to occur widely.

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UNUSUAL C_{26} POLYUNSATURATED FATTY ACIDS FROM THE MARINE SPONGE *Microciona prolifera*. ELAINE JEFFERTS, REGINALD MORALES and CARTER LITCHFIELD, Dept. of Biochemistry, Nelson Biological Labs., Rutgers University, New Brunswick, N.J. 08903.

Fatty acid analysis of the total lipids from the marine sponge *Microciona prolifera* by gas liquid chromatography on an EGSS-X column revealed two major peaks with equivalent chain lengths values 26.55 and 27.25. Each of these components was isolated as a separate band by thin layer chromatography on $AgNO_3$/silicic acid. Characterization of the two unknowns by IR spectroscopy, by NMR, and by gas liquid chromatography of the hydrogenated derivative revealed that the unknown acids were 26:2 and 26:3 containing only nonmethylene-interrupted *cis* double bonds. Reductive ozonolysis identified the 26:2 as *cis*-5,*cis*-9-hexacosenoic acid and the 26:3 as *cis*-5,*cis*-(9 or 15), *cis*-19-hexacosenoic acid. Analysis of the fatty acid composition of the four major classes of *Microciona* lipids separated by thin layer showed >40% C_{26} acids in the neutral lipids, the phosphatidylethanolamine, and the phosphatidylserine, but only 3.8% C_{26} in the phosphatidylcholine. The presence of such high levels of C_{26} acids in *Microciona* membranes must give them a unique structure.

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OCCURRENCE OF THE POLYPRENOL, DOLICHOL, IN HUMAN AND BEEF PITUITARIES. K.K. CARROLL, A. VILIM and M.C. WOODS, Dept. of Biochemistry, University of Western Ontario, London, Ont., Can. N6A 3K7.

In earlier studies on the lipid composition of human and beef pituitaries (Singh and Carroll, Lipids 5:121 [1970]), separation of the lipids by column and thin layer chromatography disclosed an unknown compound in the triglyceride fraction. Analysis by IR and NMR spectroscopy indicated a polyprenol, which was further identified as dolichol by direct comparison with an authentic sample from pig liver. Mass spectroscopy indicated that the main component contained 19 isoprene units, and analysis of the NMR spectrum showed the presence of three *trans* isoprene residues, one being the ω-terminal residue. Human pituitaries are a particularly rich source of dolichol, containing ca. 1.4 g/kg of wet tissue, mainly as the free alcohol. Beef pituitaries contained ca. 130 mg/kg, of which ca. 25% was esterified to fatty acids.

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RESIDUAL EFFECTS OF EXERCISE IN RATS. JOHN HEBERT and ALFREDO LOPEZ-S., Dept. of Medicine, LSU Medical Center, 1542 Tulane Avenue, New Orleans, La. 70112.

The metabolic effects of exercise were studied in five series of experiments using 100 adult male rats. The rats were exercised in activity cages for 8–10 weeks with food and exercise allowed ad libitum. The animals were sacrificed at the end of the exercise period, and 3 and 5 weeks after termination of exercise, to determine residual metabolic and morphological changes induced by exercise by comparison to sedentary controls. Exercised rats consumed more food (+7%), gained less weight (−5%) and had larger adrenal glands (+15%) than the controls. The exercised rats had lower serum cholesterol (−3%) and lower serum triglycerides (−30%). The activity of liver glucose-6-phosphate dehydrogenase (G-6-PD) was lower (−20%) in the exercised rats. The differences between exercise and control rats in serum cholesterol, serum triglycerides, adrenal glands weight and G-6-PD activity tend to disappear within 3 weeks after termination of the exercise and have completely disappeared after 5 weeks. These experiments provide further evidence of the effects of exercise on adrenal gland function and its possible regulatory control of metabolic changes in lipogenesis associated with exercise.

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EVIDENCE FOR THE INVOLVEMENT OF A STEROL IN UV-CARCINOGENESIS. HOMER S. BLACK and DAVID R. DOUGLAS, Dept. of Dermatology, Baylor College of Medicine, Houston, Tex. 77025.

Cholesterol γ-oxide is known to possess carcinogenic properties when administered to rodents. This compound has been shown to be formed in both human and rodent skin irradiated with UV light. The presence of such a compound with known carcinogenic properties does not, in itself, constitute sufficient evidence to implicate that compound in disease etiology. Experiments were designed, therefore, to determine whether a relationship existed between the UV-induced formation of this compound in vivo and the induction of tumors. Hairless albino mice were subjected to chronic suberythema doses of UV-radiation, dorsal skin removed at designated weekly intervals, and cholesterol γ-oxide levels determined by a combination of thin layer and gas liquid radiochromatographic analysis. No effect on cholesterol γ-oxide formation was seen until after 6 weeks chronic exposure to UV. At that time an increase in concentration occurs until about week 10, whereupon the level decreases and tumors first appear. Thus an apparent relationship exists between the formation of cholesterol γ-oxide and the onset of UV-induced tumors. These studies indicate that cholesterol γ-oxide may play some role in the development of UV-induced skin cancer.

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THE ANTIMICROBIAL ACTIVITY OF ALKYL AMINES AND AMIDES OF FATTY ACIDS. JON J. KABARA, ANTHONY J. CONLEY and JOSEPH P. TRUANT, MSU-COM, East Fee Hall, East Lansing, Mich. 48823.

Fatty acids were known to possess antimicrobial activity since 1899. In an attempt to study structure-activity relationships for fatty acids, a number of derivatives were screened. From this study and others, the wide spectrum antimicrobial action for alkyl nitrogen compound became of special interest. Therefore a more extensive study of amine and amide compounds of fatty acids was undertaken. Although both gram (+) and gram (−) organisms were affected by amine compounds, the gram-positive group was more sensitive to the antibacterial action of amines than were gram-negative organisms. The most resistant organisms were most susceptible to chain length of 10–12 carbons. On the other hand, the more susceptible organisms are affected by chain lengths 2–3 carbon atoms longer, i.e., C_{14}, C_{15}. The amide derivative of previously active fatty acids were tested. In general, the amide derivatives are less active than the amines and only the more sensitive of the gram-positive organisms are affected. The more resistant gram-positive and all of the gram-negative bacteria were not affected by amides of fatty acids. Although it is not known with any degree of certainty just what the law(s) governing antimicrobial action(s) of these compounds are, a number of mechanisms will be discussed.

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DEPRESSION OF THE LECITHIN-CHOLESTEROL ACYLTRANSFERASE REACTION IN VITAMIN E-DEFICIENT MONKEYS. HUBERT S. MICKEL, K.C. HAYES and PENELOPE L. HILL, Children's Hospital Medical Center, 300 Longwood Ave., Boston, Mass. 02115.

Vitamin E deficiency in two species of monkeys reduced the esterification of cholesterol by the plasma lecithin-cholesterol acyltransferase reaction. The reduction was apparent in ani-

mals fed a diet rich in polyunsaturated fatty acids and stripped of vitamin E. Concomitant to this in vitro measure was an alteration in the concentration of circulating polyunsaturated fatty acid cholesteryl esters. Since the plasma lecithin-cholesterol acyltransferase reaction has been shown to be dependent on sulfhydryl sites on the enzyme, it is proposed that the observed reduction in esterification of cholesterol by plasma from vitamin E-deficient monkeys is due to alteration of these sulfhydryl sites. A similar reduction in the plasma lecithin-cholesterol acyltransferase reaction has been shown by us to occur during exposure in vivo to a pure oxygen atmosphere, a condition predisposing to liquid peroxidation.

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HYOCHOLIC ACID: AN "INTERNAL STANDARD" FOR ISOLATION AND QUANTITATION OF HUMAN FECAL BILE ACIDS. M.T. RAVI SUBBIAH, Mayo Clinic, Rochester, Minn. 55901.

Methods currently available for the quantitation of human fecal bile acids do not have an "internal standard" that could be added prior to isolation procedures and would accompany the fecal bile acids through purification and quantitation. After experimenting with a number of bile acids, it was found that hyocholic acid could serve as an ideal "internal standard" for the following reasons: (a) it is not present in human feces; (b) it accompanies the bile acids during the purification procedures; and (c) it is separated from all other fecal bile acids during gas liquid chromatography (GLC). After addition of hyocholic acid (2 mg), the fecal samples were saponified, extracted, purified by thin layer chromatography (TLC), and quantitated by GLC as trifluoroacetates. The mean (±SD) recoveries of cholic-^{14}C acid and taurocholic-^{14}C acid during the extraction procedures were 91.1 ± 4.8 and 88.0 ± 1.3%, respectively. During elution procedures, the recovery of hyocholate and other bile acids exceeded 93%. The retention times of other bile acid methyl ester trifluoroacetates in relation to hyocholate were: lithocholic, 0.31; deoxycholic, 0.50; chenodeoxycholic, 0.66; hyodeoxycholic, 0.75; cholic, 1.12; and 7-ketolithocholic, 1.33. The GLC response of hyocholate was comparable with that of other bile acids. With hyocholate as an internal standard, the mean (±SD) concentration of total bile acids (in six samples processed simultaneously) was 2.69 ± 0.25 mg/g feces. It is concluded that hyocholic acid is an ideal "internal standard," allowing correction for losses during fecal bile acid analysis.

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LIPID ABSORPTION BY POLYURETHANE NETWORK POLYMERS. H.E. MARSH, JR., and C.J. WALLACE, Dept. of Chemical Engineering, South Dakota School of Mines and Technology, Rapid City, S.D. 57701.

The medicinal reduction of cholesterol levels in serum and tissue is presently accomplished by indirect means. Ion exchange polymers are employed for binding of bile acids, which are produced in the liver by the transformation of cholesterol, thus preventing intestinal reabsorption and subsequent assimilation of the bile acids. The unique aspects of the work described in this paper involve an absorption mechanism rather than ion exchange and direct removal of cholesterol in addition to bile acids. Many crosslinked polyurethane polymers were prepared and tested for lipid absorption from an isotropic micellar bile solution. In vitro absorption measurements confirmed the theory that both hydrophilic and lipophilic segments are necessary in the network polymer for significant lipid absorption from micellar bile. Both absorption rate and capacity were highly dependent on polymer formulation and size of the polymer particles. A maximum absorption of 10% lipids (based on dry polymer weight) was observed after 5 min contact with bile solution, with 59% absorption at equilibrium. For the polymers investigated, the ratio of the total absorption of water plus lipids to that of lipids alone was closely related to polymer formulation. Polymers with high hydrophilic proportions absorbed a higher percentage of water than polymers that were predominantly lipophilic. Cholesterol, lecithin and sodium cholate were confirmed in the absorbate by thin layer chromatography.

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ORAL CONTRACEPTIVE/α-TOCOPHEROL INTERRELATIONSHIPS. L. AFTERGOOD and R.B. ALFIN-SLATER, School of Public Health, University of California, Los Angeles 90024.

In previous investigations, we have observed that some of the side effects of the administration of oral contraceptive drugs to rats resemble those resulting from vitamin E deficiency. These effects include interference with reproduction, changes in lipoprotein distribution and a decrease in PUFA in various tissues. The possibility therefore exists that the administration of oral contraceptives may increase the requirement for vitamin E. Consequently we have attempted to correlate the effects of a physiological dose of Enovid E with the α-tocopherol status in the female rat. Animals were kept from weaning on the following diets all of which contained a 15% stripped corn oil: (a) Basal (no tocopherol), (b) Basal + α-tocopherol to provide 1 mg/rat/day, (c) Basal + BHT, (d) Basal with α-tocopherol given only during drug administration. At 13 weeks of age, Enovid E was orally administered at a level corresponding to 0.002 mg of mestranol and 0.05 mg of norethynodrel per day for either 4 or 28 days, at which time the rats were sacrificed and several determinations which included plasma, liver and adrenal cholesterol levels, fatty acids of lipid fractions from these tissues, α-β lipoprotein ratios, α-tocopherol levels in plasma, TBA values in liver and in adipose tissue, and hemolysis of red blood cells, were performed. The results will be discussed.

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THE EFFECTS OF (−)-HYDROXYCITRATE ON LIPID METABOLISM IN THE RAT. ANN C. SULLIVAN, JOSEPH TRISCARI, JAMES G. HAMILTON and O. NEAL MILLER, Hoffmann-La Roche Inc., 340 Kingsland St., Nutley, N.J. 07110.

(−)-Hydroxycitrate is a competitive inhibitor of ATP citrate lyase (Watson et al., Arch. Biochem. Biophys. 135:209 [1970]). This action of (−)-hydroxycitrate should reduce the acetyl CoA pool, thus limiting the availability of 2-carbon units required for fatty acid and cholesterol synthesis. We have investigated, therefore, the effects of (−)-hydroxycitrate on certain aspects of lipid metabolism in the rat under conditions of acute and chronic administration. (−)-Hydroxycitrate inhibits significantly the rate of fatty acid synthesis in liver cell-free and tissue slice systems. In vivo hepatic lipogenic rates are reduced markedly after parenteral administration. The rate of lipogenesis in liver, adipose and intestine is depressed significantly after oral administration. Of the four stereoisomers of hydroxycitrate, only (−)-hydroxycitrate decreases significantly the in vivo and in vitro rate of lipid synthesis. The chronic oral administration of a nontoxic dose of (−)-hydroxycitrate for 11–90 days causes a significant reduction in body weight gain, food consumption and total body lipid. These effects of chronically administered (−)-hydroxycitrate are observed regardless of sex, age or feeding regimen. No increase in liver size or liver lipid content occurs. Pair-feeding studies demonstrate that the reduction in food intake accounts for the decrease in weight gain and total body lipid. However in vivo rates of fatty acid and cholesterol synthesis are significantly inhibited in the (−)-hydroxycitrate-treated animals, whereas normal rates are observed in pair-fed controls and unrestricted controls. The (−)-hydroxycitrate-mediated depression of food consumption was studied in rats made hyperphagic by destruction of the ventromedial hypothalamic nuclei. These hyperphagic animals responded to chronic (−)-hydroxycitrate treatment by a reduction in food intake and weight gain similar to nonlesioned controls.

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EFFECT OF ETHANOL ON LIPID METABOLISM. CHARLES S. LIEBER, Section of Liver Disease and Nutrition, Bronx VA Hospital, 130 W. Kingsbridge Rd., Bronx, N.Y. 10468.

Both in man and in rats, ethanol abuse produces fatty liver which could not be prevented by supplementation in protein, minerals and vitamins, including choline. In rats, protein and choline deficiency potentiated the effect, whereas replacement of dietary fat by medium chain triglycerides or carbohydrates decreased the capacity of ethanol to produce steatosis. Administration of a single large dose of ethanol to rats represents a stressful condition associated with moderate hepatic accumulation of fatty acids derived from adipose tissue. By contrast, chronic ethanol administration produced more pronounced steatosis with a predominance of endogenously synthesized and, when available, dietary fatty acids. These accumulate because of decreased fatty oxidation, demonstrated both in liver slices and isolated perfused livers. Ethanol also stimulates hepatic lipogenesis. These various effects can be explained by the increase of the hepatic NADH/NAD ratio secondary to the oxidation of ethanol via the alcohol dehydrogenase (ADH) pathway. In addition there are more lasting changes in intermediary metabolism, such as increased hepatic ketogenesis which could be linked to the persistent alteration in mitochondrial function and structure found after chronic ethanol ingestion. The ultrastructural changes are also characterized by proliferation of the hepatic smooth endoplasmic reticulum, now documented by subfractionation. This led to the description of a new pathway for ethanol metabolism, the microsomal ethanol oxidizing system (MEOS). MEOS doubled in activity after ethanol feeding, whereas the activity of the cytosolic ADH decreased. The existence of MEOS may contribute to our understanding of increased cholesterol and lipoprotein synthesis. Other effects on lipid metabolism include decreases in FFA and glycerol concentrations and FFA turnover, which result from inhibition of peripheral fat mobilization by acetate, a metabolite of ethanol. In conclusion, changes in lipid metabolism secondary to alcohol ingestion are produced by hepatic generation of NADH, metabolites (such as acetate), or result from more permanent alterations in both mitochondria and endoplasmic reticulum.

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NEW DRUGS AFFECTING LIPID METABOLISM. DAVID KRITCHEVSKY, The Wistar Institute, 36th and Spruce Streets, Philadelphia, Pa. 19104.

In the last few years several promising hypolipidemic agents have been introduced. The effects of these drugs on serum lipids, cholesterol biosynthesis and cholesterol oxidation in rats and experimental atherosclerosis will be discussed. Among the compounds of interest are: pyridinolcarbamate, various linoleic acid amides, 2-acetamidoethyl (*p*-chlorophenyl) (*m*-trifluoromethylphenoxy) acetate (Halofenate), 4,4'-(isopropylidenedithio) bis (2,6-di-*t*-butylphenol) (Biphenabid), Colestipol, (2-methyl-2-[*p*-1,2,3,4-tetrahydro-1-naphthylphenoxy]) propionic acid (Melipan) and 1,1-Bis (4'-[1"-carboxy-1"-methylpropoxy] phenyl) cyclohexane. Of these drugs, Biphenabid, Colestipol and the linoleamides reduce the severity of cholesterol induced atherosclerosis in rabbits. Pyridinolcarbamate inhibits hepatic cholesterogenesis in rats. Cholesterol oxidation by rat liver mitochondrial preparations is enhanced by Melipan and Halofenate, reduced by Biphenabid and unaffected by some linoleamides.

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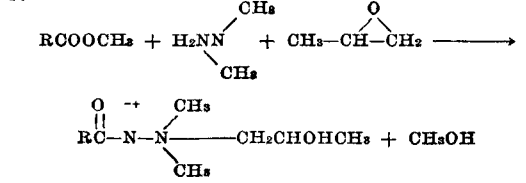
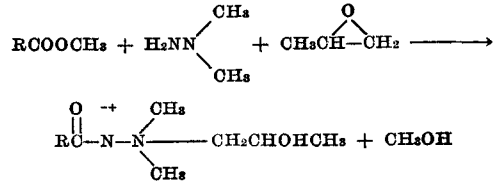
AMINIMIDE SURFACTANTS. RICHARD EGAN, EDWARD A. SEDOR and ROBERT A. GRIMM, Ashland Chemical Co., P.O. Box 2219, Columbus, Ohio 43216.

1,1-Dimethyl-1-(2-hydroxypropyl) aminimides are prepared by the reaction of an ester with *unsym*-dimethylhydrazine and propylene oxide.



Aminimides of this type were prepared from C12, C14, C16 and C18 methyl esters, and the resulting nonionic materials were found to be excellent surfactants. Properties of these aminimides to be discussed include surface and interfacial tensions, acid and alkali stability and general solution properties. Data on foam properties and detergent compatibilities will be presented, as well as the evaluations of experimental detergents. Aminimides are typically nontoxic and nonirritating, and some of this data will be presented. 1,1-Dimethyl-1-(2-hydroxypropyl) aminimides are prepared by the reaction of an ester with *unsym*-dimethylhydrazine and propylene oxide.



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PRODUCT SAFETY PARAMETERS IN SURFACTANT SYSTEM DESIGN. E.A. KNAGGS, JOHN A. YEAGER, JOSEPH R. WECHSLER, ANDREW SHULTZ and THOMAS G. BAKER, Stepan Chemical Co., Edens & Winnetka, Northfield, Ill. 60093.

The seemingly ever-increasing recent wave of consumerism and environmental protectionism movements, catalyzed by both public and governmental pressures, has caused formulators of personal care and household detergent products to consider a new dimension in the design and marketing of such products. In addition to meeting ever-increasing standards of product quality and efficacy, add a new dimension—product safety. FHSLA and similar protocol attempts to define product safety in terms of animal LD 50, Draize Eye and Primary Skin Irritation Tests. The authors explore the relationship of organic surfactant properties including foaming, wetting and C.M.C. structure and so-called "mildness" properties. Surfactant species studied include R–O–SO_3B, R–O–$(CH_2–CH_2–O)_x$ SO_3B, R^1–CH(SO_3B)–CO_2R^2, R–C_6H_5–SO_3M, R–SO_3B (olefin sulfonates) (where B includes inorganic and organic bases) and other selected types. Such basic property information is invaluable to the formulating chemist from a screening and starting point, but it is fully recognized and emphasized that it is the properties of the final formulation that are all important, and ultimately that of human experience. Examples of the effect of blending surfactant components on formulation "mildness" are presented. The limitations and reproducibility of such animal testing are recognized and discussed.

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AMPHOTERIC SURFACTANTS FROM MIXED N-ALKYLETHYLENEDIAMINES. C. BLUESTEIN, W. ROSENBLATT, J.R. CLARK and A. STEFCIK, Technical Center, 100 Bauer Dr., Oakland, N.J. 07436.

The preparation of amphoteric surfactants by the condensation of mixed N-alkylethylenediamines and unsaturated acid derivatives, particularly maleic acid esters, was studied. The reaction proceeded readily near 100 C either neat or with solvents. Saponification of the ester products yielded salts which were very water-soluble. The mono- and disodium salts of mixed N-alkyl ($C_{11.5}$ av) ethylenediamine dipropionic and diisobutyric acid (crotonic acid derived) as 1% active in water were completely soluble at all pH levels. The solutions demonstrated no perceptible isoelectric range on addition of 1–10% concentrations of sodium chloride. Data on surface tension, solubility, calcium tolerance, foam, viscosity, color and density are presented.

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SOAP-BASED DETERGENT FORMULATIONS: VI. ALKYLARYL SULFONAMIDE DERIVATIVES AS LIME SOAP DISPERSING AGENTS. R.G. BISTLINE, JR., W.R. NOBLE and W.M. LINFIELD, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

Alkylbenzenes, such as industrial "detergent alkylates," as well as pure 1-phenylalkanes whose side chain lengths varied from C8 to C_{12}, were converted into the corresponding alkylbenzenesulfonyl chlorides with chlorosulfonic acid. Surface active sulfonamides were obtained from the reaction of the sulfonyl chlorides with various low molecular aminosulfonic acids, such as N-methyltaurine, or with aminoalkyl esters of sulfuric acid such as 2-aminoethyl hydrogen sulfate. The hydrolytic stability of the resulting surface active sulfonamide derivatives was investigated. As expected, the sulfonates were quite resistant to acid or alkaline hydrolysis, while the sulfates were more susceptible to hydrolysis. Hydrolytic stability of the sulfates was poorer under alkaline conditions than under acidic conditions. All of the compounds were excellent lime soap dispersing agents giving Borghetty-Bergman values in the range of 6 to 10. The compounds were evaluated for detergency either alone or formulated with tallow soap, or formulated with tallow soap and sodium silicate (Na_2O/SiO_2 1:1.6). The derivatives of the pure hydrocarbons that gave the best overall detergency were those of 1-phenyldecane; those of 1-phenyldodecane were just slightly inferior, whereas those of 1-phenyloctane exhibited poor detergency. This ranking was observed when the compounds were tested alone as well as when formulated. The sulfonamide derivatives of the "detergent alkylate" type of alkylbenzenes exhibited excellent detergency characteristics and showed substantial potentiation of detergency when mixed with soap or with a soap-sodium silicate blend. The detergency performance of these formulated detergents was equal to that of commercial household detergents in single wash tests.

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SOAP-BASED DETERGENT FORMULATIONS: VII. DERIVATIVES OF ALKYLBENZOYLSULFOPROPIONIC ACIDS AS LIME SOAP DISPERSING AGENTS. WILLIAM N. MARMER and W.M. LINFIELD, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

A series of alkylbenzoylsulfopropionic acid derivatives were prepared from commercial detergent alkylates (linear alkylbenzenes) as well as from individual alkylbenzenes via the Friedel-Crafts reaction of the alkylbenzenes with maleic anhydride. The resulting alkylbenzoylacrylic acids were esterified with methanol and other alcohols, and the esters thus obtained were sulfonated by bisulfite addition across the double bond. Further reaction of the methyl esters with amines such as ethanolamine, diethanolamine or 3-(N,N-dimethylamino)propylamine resulted in amides. The amides from the latter amine were converted to the corresponding amine oxides with hydrogen peroxide. All of the above derivatives of decyl- or dodecylbenzene were found to be good lime soap dispersing agents giving Borghetty-Bergman values in the 6–10 range. The derivatives were formulated with tallow soap, glassy sodium silicates, and sodium carboxymethylcellulose to give detergent compositions whose performance characteristics in single wash tests were similar to those of commercial phosphate built detergents.

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THE BIOLOGICAL BEHAVIOR OF SOME SOAP-BASED DETERGENTS UNDER AEROBIC AND MICROAEROPHILIC CONDITIONS. E.W. MAURER, T.C. CORDON, J.K. WEIL and W.M. LINFIELD, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

The biodegradability of tallow soap, three soap-based detergent formulations and their component lime soap dispersing agents—sodium methyl α-sulfotallowate (TMS), sulfated N-(2-hydroxypropyl) tallowamide (TAM), and sodium N-methyl N-(2-sulfoethyl) tallowamide (Igepon T)—was determined under aerobic and microaerophilic conditions. Linear alkylbenzenesulfonate (LAS) was used as the reference standard. Both sewage and river water microorganisms were used as the sources of inoculum. The course of biodegradation was followed by loss of carbon and methylene blue active substance (MBAS), and by increase in turbidity and surface tension. Carbon analysis for soap in solutions containing Ca^{++} and Mg^{++}, which would precipitate soap, was performed by an improved technique using the disodium salt of ethylenediamine tetraacetic acid. Invariably, a decrease in carbon content was accompanied by an increase in turbidity and surface tension. Also, loss in MBAS was concurrent with an increase in turbidity and surface tension of the degrading solutions of the detergent. Soap cannot be determined as MBAS because of the low pH of the test. Soap and the built soap formulations degraded under aerobic and microaerophilic conditions, while LAS, used as a control, did not degrade in the microaerophilic tests. Preliminary toxicity data on mammals and fish indicated that these detergents are as safe as conventional commercial detergents.

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TEST VARIABLE EFFECTS IN DETERGENCY TESTING WITH SEBUM-SOILED CLOTHS. TED P. MATSON and STEPHEN E. MCGUIRE, Continental Oil Co., P.O. Drawer 1267, Ponca City, Okla. 74601.

Reproducibility in detergency test procedures depends upon a myriad of test variables. Many of these variables have received little attention, with their contribution to error being avoided by such techniques as using only cloths from a single sebum-soiling batch in making product comparisons. This study evaluates many of the cloth variables in Terg-O-Tometer detergency testing using sebum-soiled cloths. Some of the variables studied were age of soiled cloth, soil load per washing and cloth soil density. Evaluations also included the effects of washing cotton, permanent press and dacron simultaneously vs. individually. Effects of the variables upon detergency were determined by regression analysis.

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PROCESSING OF EDIBLE PEANUT FLOUR. JAMES L. AYRES, LEWIS L. BRANSCOMB and GLENDA M. ROGERS, Gold Kist Research Center, P.O. Box 388, Lithonia, Ga. 30058.

Edible peanut flour and grits have been produced by a commercial prepress, solvent extraction method. The finished flour exhibits excellent extrusion-expansion characteristics for use in both cereal and snack food items. Soluble carbohydrate profile indicates peanut flour is lower in raffinose and stachyose than commercial soy flour. The bland flavor and light tan color facilitates incorporation of peanut flour and grits into a wide range of food products.

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PROCESSING FOR NONRUMINANT FEED MARKETS. C.R. RATHBONE, Ranchers Cotton Oil, Box 248, Fresno, Calif. 93721.

Processing methods can be controlled to provide a cottonseed meal that is improved nutritionally and substantially free from toxic principles. In so doing, cottonseed meal can be fed beneficially to cattle, poultry, swine and even trout. It is therefore possible to feed cottonseed meal to animals and poultry up to 10% of their dietary levels without harmful effects like minimizing their growth, reducing their bone marrow, harming their spleens or discoloring their egg yolks. It is, however, necessary to prove to those who might use the meal that the feeding is economically sound and as productive as other meals.

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SYSTEM FOR THE PRODUCTION OF HIGH AND LOW PDI EDIBLE EXTRACTED SOYBEAN FLAKES. E.D. MILLIGAN and J.F. SURIANO, EMI Corp., 3166 Des Plaines Ave., Des Plaines, Ill. 60018.

A standard Flash Desolventizing system has been combined with horizontal agitated meal stripping and cooking vessels operating at atmospheric pressure to provide an integrated system for the production of high, intermediate or low PDI edible soybean flakes from extracted solvent-wet flakes. Flash Desolventizing removes most of the hexane in the wet flakes by evaporation at low temperature in a turbulent stream of superheated hexane vapor. The small remaining hexane quantity is removed in a "stripping" process capable of producing the full range of PDI values in the flakes by treating the flash-desolventized flakes with either dry superheated steam or wet saturated steam under carefully controlled conditions of steam temperature, pressure, flow rate and moisture content. The products are light colored, with little production of fine particles.

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IMPROVED SYSTEM FOR BULK MEAL STORAGE. E.D. MILLIGAN and J.F. SURIANO, EMI Corp., 3166 Des Plaines Ave., Des Plaines, Ill. 60018.

A system was installed for the bulk storage of soybean meal and operated in a manner designed to eliminate the problems commonly associated with such storage, such as hangups and

damage to tanks due to dropping of arched meal. The final system design and operation was based upon the results of study of a large number of operating soybean meal storage systems and their problems. The primary consideration in successful storage of meal was found to be the condition of the meal itself and its tendency to consolidate when stored fresh from production. When the meal stored in this system was disturbed at regular intervals, this tendency was overcome, and the meal moved freely and uniformly without arching. The design and operating features of the system are described.

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DEHULLING COTTONSEED AND SEPARATING KERNELS AND HULLS: COMPARISON OF SEVERAL VARIETIES OF SEED. S.P. CLARK, L.R. WIEDERHOLD, C.M. CATER and K.F. MATTIL, Oilseed Products Div., Food Protein R&D Center, Texas A&M University, College Station 77843.

The proteinaceous components of cottonseed can be converted into several different forms for use in human food. All of them require nearly complete separation of kernels and hulls. In research on improving separation processes, seven multiton lots of cottonseed were processed through pilot size commercial-type dehulling and kernel-hull separating machinery. The machinery was operated to produce the cleanest separations possible. Each lot of seed was from a different variety of cotton. One lot was an experimental glanded and two lots were experimental glandless varieties. The glanded seed and one of the glandless varieties were weak hulled. Three lots were commercial glanded varieties. One lot was gin-run seed. For six of the lots, seed were delinted to two levels of ca. 7.0 and 2.5% residual linters, and separate dehulling runs were made on seed of each level. The weak hulled seed were the only lots showing any important differences in dehulling characteristics. They produced much higher yields of coarse kernels than the other lots. In terms of nearly pure kernels, good results were obtained with all lots. Yields of kernels ranging from 72 to 96% of the total kernels from each seed were concentrated into a product which contained less than 0.5% hulls.

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A PROGRESS REPORT ON THE PRODUCTION OF FOOD-GRADE COTTONSEED PROTEIN BY THE LIQUID CYCLONE PROCESS. J.M. RIDLEHUBER, Plains Cooperative Oil Mill, P.O. Box 1889, Lubbock, Tex. 79408, and H.K. GARDNER.

Plains Cooperative Oil Mill has scheduled for completion in late spring a commercial installation for the Liquid Cyclone Process (LCP) with an initial capacity of 25 tons of cottonseed flour a day. Designed by Engineering Management Inc. (EMI), Des Plaines, Ill., the installation includes some significant engineering innovations in meats preparation, extraction, desolventization, and product packaging. Design criteria included features necessary to produce a food-grade product. This commercial installation will make possible for the first time a supply of high-protein, low-gossypol flour from glanded cottonseed. While this plant was being constructed, ca. 8000 lb of edible flour from glanded cottonseed was produced by the LCP in the SRRL pilot plant and is being evaluated for use in many food preparations. Cottonseed flour produced by this process was approved as a Food Additive by FDA as of July 13, 1972. The original process has been simplified, and yields of flour have been increased without impairment to its high quality. In addition to its application to glanded cottonseed, the LCP has been tested at SRRL on a pilot plant basis to yield from glandless cottonseed two important protein products: a 70% protein flour and a 50% protein meal. Grain Processing Corp., Muscatine, Iowa, is evaluating flour supplied from SRRL pilot plant operations. In April 1972, this Corporation negotiated an agreement to market the total production of flour from Plains Cooperative Oil Mill.

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AQUEOUS EXTRACTION: AN ALTERNATIVE OILSEED MILLING PROCESS. C.M. CATER, R.D. HAGENMAIER, K.C. RHEE and K.F. MATTIL, Oilseed Products Div., Food Protein R&D Center, Texas A&M University, College Station 77843.

Oil can be removed from oilseed materials by a process that consists of an aqueous extraction of the comminuted seed, followed by a centrifugal separation which divides the aqueous extract into oil, solid and aqueous phases. The protein may be recovered in the solid or aqueous phase, depending on the selected conditions. Unit operations of this process are grinding, solid-liquid separation, centrifugation and drying of products. Aqueous extraction has been applied, to date, to coconuts and peanuts. For coconuts, a procedure has been developed to recover 93% of the oil and 91% of the protein. The major protein product is 25% protein and low in fiber with many properties similar to dried skim milk. The estimated production cost of this product is 16¢/lb. The particular advantage of aqueous extraction for coconut is the separation of protein from fiber which is not achieved by conventional processing. For peanuts, the recovery of oil was 89% and protein 92% for the concentrate procedure, whereas the corresponding values for the isolate procedure were 86% and 89%, respectively. The costs of production were estimated as 17¢/lb of concentrate (67% protein) and 28¢/lb of isolate (89% protein). Aqueous extraction offers several advantages over conventional solvent extraction—a smaller economic unit allowing lower initial capital outlay; safer operation; capability of discontinuous operation; and production of a variety of products with minimum protein denaturation. Another advantage of aqueous processing is the capability for utilization of certain chemicals to remove or inactivate undesirable substances. In the case of peanuts, hydrogen peroxide and sodium hypochlorite have proved very satisfactory for detoxication of aflatoxins. Aqueous processing has the potential for application to a variety of other oilseeds.

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CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC ANALYSIS OF LIPIDS FROM BLOOD PLASMA, SERUM AND ERYTHROCYTES. GARY J. NELSON, Bio-Medical Div., Lawrence Livermore Lab., P.O. Box 808, Livermore, Calif. 94550.

This presentation will review the techniques used in the author's laboratory for the past several years for the systematic investigation of blood lipids in a wide variety of animal species. The plasma and cells are separated and the cells washed to remove residual plasma lipids. The lipids are extracted with chloroform-methanol 2:1 v/v. Data will be presented on the completeness of the extraction procedure and on the ratio of solvent to volume of cells or plasma for optimum extraction. The lipid extracts are purified before further analysis on Sephadex columns. The lipid classes are then separated using both one and two dimensional thin layer chromatography (TLC). One dimensional TLC is used for neutral lipids, glycolipids and sometimes phospholipids. Details of the TLC, including the method of preparation of the plates, type of absorbent and various solvent systems, will be described. The lipids are analyzed by spectrophotometric methods after their separation on the TLC plate, using specific color reactions for each lipid class, although lipid classes are not analyzed in mixed groups. The fatty acids associated with each individual lipid class are also analyzed after separation by TLC, using capillary-column-gas chromatography. The advantages and disadvantages of these procedures will be discussed and typical data obtained using these techniques will be presented, along with a statistical treatment of the data for repetitive analysis. These techniques are intended primarily for the research laboratory rather than the clinical laboratory and are not always either rapid or simple.

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GAS CHROMATOGRAPHIC ANALYSIS OF NATURAL MIXTURES OF COMPLEX LIPIDS. A. KUKSIS, Banting & Best Dept. of Medical Research, University of Toronto, 112 College St., Toronto 101, Ont., Can.

The development of methods for direct gas chromatographic analyses of natural lipid mixtures is a logical result of successful resolution of the individual components. The separations are best carried out by temperature programing (100–375 C) on short (50–120 cm) columns containing a nonpolar liquid phase (1–3% methyl silicone), which allow adequate resolution and recovery of both high (triglycerides) and low (free fatty acids) molecular weight components. The separations are based on differences in carbon number and are applicable to most mixtures of neutral lipids, as well as to lipids that can be converted into neutral substances prior to admission to the gas chromatograph. For the latter purpose, silylation (acids and alcohols), acetylation (alcohols), silolysis or acetolysis (phosphoglycerides and sphingomyelins) and digestion with phospholipase C (phosphoglycerides) have proved satisfactory. Substitution of a thin film of polyester for the silicone liquid phase allows resolutions based on both molecular weight and degree of unsaturation. Thus free fatty acids, monoglycerides, sterols and diglycerides can be resolved by temperature programing (100–300 C) of dual columns (180 cm) containing silicone-polyester packings (3% EGSS-X or CHDMS). The acids must be methylated or silylated, and the alcohols either silylated or acetylated prior to chromatography. The gas chromatographic results are readily quantitated by means of suitable internal standards and can be obtained on 10–50 μg of total lipid. The technique has been employed extensively in the determination of the lipid profiles of whole serum or plasma, as well as of the component lipoproteins. Other applications as well as eventual automation are obvious.

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LIPOPROTEIN PROFILE OF HUMAN BLOOD. R.B. ELLEFSON, BARBARA JIMENEZ and FUAD MANSOUR, Mayo Clinic, Rochester, Minn. 55901.

Paper electrophoresis of blood lipoproteins and the staining of the electrophoregrams have been improved and applied, together with preparative ultracentrifugation to the analysis of 60,000 specimens from more than 36,000 persons. Many of the specimens were analyzed by other methods in addition—electrophoresis in cellulose actate, agar, agarose and/or polyacrylamide, immunodiffusion and immunoelectrophoresis. Our survey has shown that the lipoprotein profile of human blood can include components of at least the following forms: VLDL of five forms—β, pre-β, two α_2 forms and chylomicrons; LDL of five forms—three variants with β_1 electrophoretic mobility, one α_2 form (rare) and one α_1 form (liver disease); and HDL of two major forms—α_2 (large aggregate) and α_1 (smaller aggregate 6–10 recognizable components). Electrophoregrams were developed with 10 μl serum or lipoprotein fraction from serum; barbital-sodium barbital at pH 8.6 was used as the buffer; the paper strips (Whatman 3 MM) were presoaked in buffer that contained bovine serum albumin; and electrophoregrams were stained with oil red O or sudan black B in aqueous acetone instead of the customary aqueous alcohol. In our experience, electrophoresis on paper and electrophoresis in agarose have yielded similar results.

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ETHER-LINKED GLYCEROL LIPIDS: DETECTION AND IDENTIFICATION OF LABELED INTERMEDIATES AND PRODUCTS FORMED IN ENZYMIC SYSTEMS. M.L. BLANK and FRED SNYDER, Medical Div., Oak Ridge Associated Universities, Oak Ridge, Tenn. 37830.

Methods used in the isolation and analysis of various lipid intermediates and products formed during the metabolism of 0-alkyl and 0-alk-1-enyl glycerolipids from labeled substrates will be described. These methods include thin layer chromatography, gas liquid chromatography and mass spectrometry used in conjunction with chemical and enzymic procedures.

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SEPARATION AND IDENTIFICATION OF PLANT LIPIDS BY THIN LAYER CHROMATOGRAPHY AND COMPLEMENTARY TECHNIQUES. MARIUS LEPAGE, Dept. of Biochemistry, and Lipid Research Center, Medical School, Laval University, Québ., Can.

In view of the difficulties encountered in the analysis of the plant lipid complex, a rapid thin layer chromatographic procedure, which allows complete separation and identification of all lipid classes, has been developed. Plant and animal neutral lipids, phospholipids, glycolipids, sterol glycosides, pigments and other related minor components can be separated. In this paper, one dimensional and two dimensional separations, solvent systems, developments, methods of detection and identification, collection and elution of the spots, and argentation thin layer chromatography of fatty acids will be discussed. Reference will be made to coupling with gas liquid chromatography.

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Fe REQUIREMENT FOR DECARBOXYLATION OF ALPHA-HYDROXY LONG CHAIN FATTY ACIDS. JAMES F. MEAD

and ROBERTA S. HARE, Lab. of Nuclear Medicine and Radiation Biology, 900 Veteran Ave., Los Angeles, Calif. 90024.

Alpha oxidation or one-carbon degradation of long chain fatty acids has been shown to occur in mammalian brain. We have studied the decarboxylation step of this pathway using [1-^{14}C]-labeled tetracosanoic acid. The reaction requires the microsomal and supernatant fraction along with O_2 and Fe^{2+}. There is recent evidence to suggest the supernatant factor is ascorbic acid. This report will deal with the finding of ferrous ion as a cofactor. As isolated, the system does not show an absolute requirement for ferrous ion. The addition of the ion does increase the activity 30–50%, depending on the preparation. However decarboxylation is inhibited totally by the addition of EDTA or 1,10 orthophenanthroline. This inhibition can be overcome by Fe^{2+} or Fe^{3+} ions. Of the metals tested for activity, Ca^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and Zn^{2+} seemed to be inhibitory. Ca^{2+} and Mg^{2+} had no reactivation activity. Fe^{3+} ion, we feel, is being reduced immediately upon addition to the reaction mixture and then oxidized in the course of the reaction to ferric ion.

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INFLUENCE OF DIETARY FATTY ACIDS ON LIVER MICROSOMAL GLUCOSE-6-PHOSPHATASE IN VITRO. GUSTAV GRAFF, S.J.C. YEH and F.A. KUMMEROW, Burnsides Research Lab., University of Illinois, Urbana 61801.

Male weanling rats from the Holtzman strain were fed ad libitum three different types of diets: a) fat deficient diet; b) laboratory chow diet; c) a diet containing *trans* fatty acid. After 3 months the animals were killed, the livers excised, and used for microsomal preparation. The kinetic aspects of glucose-6-phosphatase reaction and the relevant activation energies were investigated. Changes in kinetic constants and activation energies resulting from these dietary manipulations were observed. Furthermore, differences in microsomal phospholipid content were found.

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COMPARISON OF TRIACYLGLYCEROL SPECIES ISOLATED FROM THE MAJOR LIPOPROTEIN PARTICLES OF INDIVIDUALS WITH TYPE IIa AND NORMAL LIPID PROFILES ALTER CONTROLLED DIET. DENNIS T. GORDON and ROBERT G. JENSEN, Dept. of Nutritional Sciences U-17, University of Connecticut, Storrs 06268.

After screening a volunteer male population, individuals with a type IIa and normal lipoprotein profile were selected and placed on a strictly regulated diet for 14 days. The first 7 days consisted of a diet low in cholesterol and with an increased unsaturated to saturated fat ratio. During the second 7 day period, cholesterol intake was increased and the ratio of unsaturated to saturated fat was decreased. At the end of each 7 day period, the triacylglycerol of the major lipoprotein particles were isolated and stereospecifically analyzed. The major lipoprotein particles isolated by preparative ultracentrifugation include chylomicrons, and particles of density < 1.006, < 1.063 and 1.210 g/ml. The relationships between the circulating triacylglycerol species of normal and type IIa individuals will be discussed from the standpoint of predominant species, biosynthesis and degradation.

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CATHARTIC ACTION OF LESQUERELLA AID VERNONIA OILS AND RELATED DERIVATIVES. M.S. MASRI and A.N. BOOTH, Western Regional Research Lab., ARS, USDA, Berkeley, Calif. 94710.

We have extended our previous observations on the relation of cathartic activity to structural modification of ricinoleic acid and the specific configuration of castor oil responsible for its action, to similar observations on the related lesquerella, dimorphotheca and vernonia oils and derivatives of these. The results corroborate and compliment our previous observations with castor oil and derivatives. The samples tested (in the rat) included: lesquerella oil, methyl lesquerolate, O-acetyl methyl lesquerolate, dimorphotheca oil, vernonia oil, trivernolin and methyl 12,13-dihydroxyoleate; castor oil and corn oil were used for comparison (cathartic and noncathartic, respectively).

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EFFECTS OF CHRONIC ALCOHOL INGESTION ON THE NADPH-CATALYZED PRODUCTION OF LIPOPEROXIDES. RONALD C. REITZ, Dept. of Biochemistry, University of North Carolina, Chapel Hill 27514.

TPNH oxidase or NADPH cytochrome *c* reductase has been shown to be linked to the microsomal production of lipid peroxides in the liver. Our studies on the effects of chronic alcohol ingestion on liver lipids have revealed that chronic ingestion caused an increase in the activity of this mixed function oxygenase compared to either glucose-fed or chow-fed controls. This increased activity of TPNH oxidase also led to an increase in the production of lipoperoxides. Lipid peroxides were measured in two ways. First, the rates of conjugated diene formation were measured by extracting the lipids and reading the change in absorbance at 232 nmeters. Second, the rate of malondialdehyde formation was measured by the thiobarbituric acid assay technique. The results of these studies showed that there was an increase in the rates of formation of both malondialdehyde and conjugated dienes in the alcohol-treated animals. The effects of temperature on the production of malondialdehyde were measured in the three groups of animals. The temperature maximum was found to be 33 C. There was a two-fold increase in the rate of malondialdehyde formation over a 12 C increase in temperature. When these data were subjected to Arrhenius Plot analyses, it was found that the energy of activation for the formation of malondialdehyde was 14,260 calories in the ethanol-treated animals. The two control groups had considerably lower energies of activation: 9510 calories for the glucose-control, and 9260 calories for the chow-control. The oxidation of NADPH initiates the sequence of reactions which ultimately leads to the production of lipid peroxides. On the other hand, the formation of both malondialdehyde and conjugated dienes are reactions that occur after a lipid peroxide has been formed. Therefore the consumption of molecular oxygen, which is one of the initial reactions in peroxide formation, was measured in the microsomal TPNH oxidase-catalyzed peroxidation system. This data is correlated with the activity of the TPNH oxidase and the formation of malondialdehyde and conjugated dienes. Also the effects of temperature on oxygen consumption in the three groups of animals will be reported.

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MECHANISMS OF PEROXIDATION HEMOLYSIS OF ERYTHROCYTES FROM VITAMIN E-DEFICIENT RATS AND RABBITS. MYRA O. BARKER, LYNDA HORN, GWEN REED and MYRON BRIN, Hoffmann-LaRoche Inc., Dept. of Biochemical Nutrition, 340 Kingsland Street, Nutley, N.J. 07110.

Erythrocytes (RBC) from vitamin E-deficient rats hemolyze readily in the presence of hydrogen peroxide (HP), glucose oxidase-glucose (GO-G) or dialuric acid (DA); RBC from vitamin E-deficient rabbits are susceptible to HP but are relatively resistant to GO-G and DA. We have reported (Fed. Proc. 31:694 [1972]) that RBC from vitamin E-deficient rats, incubated with GO-G 30 min at 37 C and placed in the dialysis membrane of the Fragiligraph produce a leftward shift of the fragility curve from control values (peroxidative hemolysis). RBC from vitamin E-deficient rabbits under the same conditions do not produce a shift of the Fragiligram unless rabbits were given daily oral doses of arachidonic acid for several weeks prior to testing. In further experiments, we pretreated RBC from vitamin E-deficient and supplemented rats and rabbits with GO-G for 15 min at 37 C, washed cells free of GO-G and then studied susceptibility to phospholipases A and C. Rat RBC pretreated with GO-G showed increased susceptibility to both phospholipases, indicating that partial peroxidation of membrane phospholipids may release both polar and nonpolar parts of the molecule from the membrane matrix. Rabbit RBC, however, showed increased susceptibility only to phospholipase A, indicating that only the nonpolar end of the molecule may be released from the membrane matrix by partial peroxidation. These findings indicate that the basic mechanisms of hemolysis of rat and rabbit RBC by peroxide-generating systems are different.

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STERCULIC ACID AS A COCARCINOGEN WITH AFLATOXIN IN RATS. PENELOPE WELLS, LILLA AFTERGOOD and ROSLYN ALFIN-SLATER, UCLA School of Public Health, 405 Hilgard Ave., Los Angeles, Calif. 90024.

Investigations on trout have shown that the cyclopropenoid fatty acids, which occur naturally in small amounts in cottonseed, are powerful cocarcinogens when fed in conjunction with aflatoxin. Attempts to document these findings in mammals, i.e., rats, have not been conclusive. In addition, earlier work in this laboratory has indicated that in livers of rats fed aflatoxin, monounsaturated fatty acids increase. Since sterculic acid is known to block the formation of the monounsaturated oleic acid, it was decided to investigate the effects of sterculic acid on lipid metabolism and tumor formation as a result of aflatoxicosis in rats. Since on fat-free regimens rats use oleic acid to form the more highly unsaturated fatty acids, male weanling rats were placed on fat-free diets to which the following additions were made: (a) Basal diet (no supplements); (b) 1.7 ppm aflatoxin B_1; (c) 200 ppm sterculic acid; (d) 1.7 ppm aflatoxin B_1 + 200 ppm sterculic acid. The rats consumed these diets for 3 months and were thereafter maintained on the basal diet until sacrifice at 7 months. The most pronounced inhibition of growth and severe liver pathology was observed in group IV. In addition, previously unobserved pathological changes were observed in kidneys of some of these group IV rats. Livers were larger both absolutely and as per cent body weight. Plasma cholesterol levels were elevated as a result of aflatoxin administration. Sterculic acid did not affect these findings. Some of the changes observed as a result of aflatoxin administration, in the fatty acids of sterol esters and triglycerides, were nullified by sterculic acid. These findings indicate that sterculic acid modifies response of the rat to aflatoxin on a fat-free regimen.

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NEW MAJOR METABOLITE OF AFLATOXIN B_1 IN MONKEY LIVER. M.S. MASRI, W.F. HADDON and R.E. LUNDIN, Western Regional Research Lab., ARS, USDA, Berkeley, Calif. 94710, and D.H.P. HSIEH.

Incubation of monkey liver microsomal fraction with crystalline aflatoxin B_1 resulted in conversion to aflatoxin M_1 and a new major metabolite, for which we propose the name aflatoxin Q_1. In a preparative experiment, both aflatoxins M_1 and Q_1 were isolated in crystalline form, and the structure of aflatoxin Q_1 was identified on the basis of UV, IR, mass and NMR spectra as an isomer of aflatoxin M_1 with the hydroxyl at the position α or β to the carbonyl of the cyclopentenone ring. The extent of the in vitro conversion was ca. 1% to M_1, but a remarkable 30–50% conversion of the added aflatoxin to Q_1 in different experiments, even when high substrate concentrations were employed (0.5–1 mg B_1 per 1g liver equivalent). Although aflatoxin Q_1 was also observed in similar preparations from rat and chicken liver, the extent of conversion was much lower (only 1–2%).

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DIMETHYLAMINE AND NITRITE FEEDING EXPERIMENTS IN RATS. KYU Y. LEE, The Eppley Institute, University of Nebraska Medical Center, 42nd and Dewey Ave., Omaha 68105.

No one would deny the existence of amines and nitrites in our environment and in the food (including water and air) we consume daily. Furthermore no one would deny formation of carcinogenic nitroso compounds by these chemicals in our environment and in our bodies under certain conditions. Finally, it is a proven experimental fact that some nitroso compounds are potent carcinogens in experimental animals. Because of these facts a great many experiments are carried out, and some have postulated a link between etiology of certain cancers and nitroso compounds. However in feeding experiments one must be cautious to observe and make record of individual rat performance rather than use a pool of rats in a cage or group. For example, some rats would rather go without eating and drinking than eat the food or water containing nitrites or a combination of nitrites and amines. As a result, one would find some animals showing no ill effect from these chemicals not because they are not toxic or carcinogenic but because the animals the investigator examined did not consume any of the chemical(s) given and assumed to be eaten. Therefore to report the test compound(s) not toxic or carcinogenic would not be correct. A survey of each individual rat's starting and daily body weights, daily food and water consumption with dimethylamine, nitrite and a combination of the two showed wide variations.

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HYDROGENATION OF SOYBEAN OIL WITH COPPER CHROMITE CONTAINING SMALL AMOUNTS OF NICKEL CATALYST. K.J. MOULTON and R.E. BEAL, Northern Regional Research Lab., ARS, USDA, 1815 N. University St., Peoria, Ill. 61604.

Soybean oil was partially hydrogenated with mixed commercial copper-chromite and nickel catalysts. The effects of small amounts of nickel on reaction rates, linolenate-linoleate reaction selectivity and conjugated diene formation were determined at copper-chromite to nickel ratios of 2000, 1000, 500, 100 and 50:1, and at catalyst concentrations in the oil of 1.0, 0.5 and 0.25%. The presence of nickel in all concentrations increased the rate of hydrogenation substantially. At copper-chromite to nickel ratios of 2000, 1000 and 500:1, only a small decrease in selectivity ($K_{1e}/_{1o}$) resulted, compared with copper-chromite alone. At these same ratios when linolenate in the hydrogenated oils is zero, their iodine values were 108–110 compared to 111 for copper chromite and less than 80 for nickel alone.

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VACUUM EQUIPMENT: EFFECT ON DEODORIZER DISTILLATES. JAMES R. MORGAN, Elliott Co., 2904 Woodburn Ave., Cincinnati, Ohio 45206.

Deodorization of edible fats and oils produces distillates that can be profitably recovered by use of a scrub cooler when used in conjunction with the normal vacuum equipment as applied to semicontinuous deodorizers. It is well known that the quality and quantity of distillates recovered depends primarily on the type of oil being deodorized, as well as the design of the deodorizer itself. However the quality and quantity of distillates recovered can also be affected by changes in important design parameters of the ejector and scrub cooler equipment. Thus it may be well to examine changes in operating pressures, operating temperatures, cooling water temperatures, steam volume loading and temperature of the scrubbing medium as it affects the recovery and saleability of the deodorizer distillates.

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EFFECT OF LIQUID FAT ON MELTING POINT AND POLYMORPHIC BEHAVIOR OF COCOA BUTTER AND A COCOA BUTTER FRACTION. N.V. LOVEGREN, M.S. GRAY and R.O. FEUGE, Southern Regional Research Lab., ARS, USDA, 1100 Robert E. Lee Blvd., P.O. Box 19687, New Orleans, La. 70179.

The polymorphic behavior of cocoa butter and a high-melting fraction of cocoa butter were investigated by differential scanning calorimetry. The effect of liquid fat on melting point and the polymorphic behavior were investigated using six mixtures: (a) 88.3% cocoa butter and 11.7% of a low-melting fraction of cocoa butter; (b) 90% cocoa butter and 10% olive oil; and (c) mixtures of a high-melting fraction of cocoa butter and olive oil containing 10, 20, 30 and 50% olive oil, respectively. Five different polymorphs were found for cocoa butter and six for the high-melting fraction. The melting points for cocoa butter and the high-melting fraction were 35 and 38 C, respectively. Addition of the low-melting fraction to cocoa butter reduced the observable polymorphs to four and the melting point to 32.5 C. Ten per cent olive oil in cocoa butter reduced the observable polymorphs to three and the melting point to 31.5 C. Similarly, 10% olive oil in the high-melting fraction of cocoa butter reduced the observable polymorphs to three and the melting point to 37 C. Amounts of 20, 30 and 50% olive oil in the high-melting fraction reduced the polymorphs to two and the melting point to 34.5, 33 and 32 C. Possible explanations for the observed polymorphic behavior are advanced. Changes in the rates of tempering of cocoa butter and the high-melting fraction resulting from the addition of various amounts of liquid fat are discussed.

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EFFECT OF FEEDING "PROTECTED" SAFFLOWER OIL ON RECOVERY OF FORMALDEHYDE IN MILK, CHEESE AND MEAT. JOEL BITMAN, T.R. WRENN, H.K. GOERING, L.P. DRYDEN and L.F. EDMONDSON, Animal Physiology & Genetics Institute, ARS, USDA, Bldg. 161, ARC-East, Beltsville, Md. 20705.

Milk, cheese and meat with high levels of polyunsaturated fatty acids may be of utility in the dietary management of blood lipids in cardiovascular diseases. Milk and meat fat with high levels of C 18:2 were produced by feeding cows and veal calves a supplement containing a formaldehyde-treated blend of safflower oil and caseinate (SOC-F). The formaldehyde-caseinate coating protects the oil against ruminal hydrogenation. To determine whether formaldehyde was transferred from the supplement to milk, cheese and meat, samples of these products and of the feed supplement were hydrolyzed with phosphoric acid and distilled. The formaldehyde released was determined by the chromotropic acid color reaction (sensitive to 0.5 ppm in milk). Lots of the SOC-F supplement contained from 0.34 to 0.94% formaldehyde. Cows readily consumed the SOC-F supplement. There were no apparent effects on health, body weight or milk production related to the ingestion of formaldehyde. Milk from cows that ingested 800 g/day of SOC-F containing 4 g formaldehyde for 4 months had no demonstrable formaldehyde. The determinations showed no significant differences between formaldehyde levels in milk, cheese and meat from animals fed SOC-F and similar products from animals which were not fed formaldehyde-containing feed. Under the conditions of our experiments and feeding trials, there was no evidence that formaldehyde is transferred into milk or muscle tissues of animals receiving dietary supplements containing formaldehyde.

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FLUORESCENT PRODUCTS OF LIPID PEROXIDATION. V.G. MALSHET and A.L. TAPPEL, Dept. of Food Science and Technology, University of California, Davis 95616.

The structural requirement for fluorescence in Schiff bases was defined. Aldehydes and amines were reacted and the structures of the Schiff bases N-hydroxyethyl-1-imino-2',4'-hexadiene (I), N-1-benzal-2-hydroxyaminoethane (II) and N-1-benzal-2,2'-hydroxyaminoethane (III) were established by elemental analysis, IR spectral analysis and mass spectral analysis. The structures of N-alanyl-2-hydroxy-naphthylidine (IV) and N,N'-dileucinyl-1-amino-3-iminopropene (V) had been established previously. III, IV and V were fluorescent compounds, and I and II were not. The results of these analyses suggest that an electron-donating group in conjugation with an imine is the structure required for fluorescence. In addition to fluorescence spectral characteristics, the effect of pH and chelation on the fluorescence yield was used to characterize 1-amino-3-iminopropene compounds which occur during in vivo and in vitro lipid peroxidation.

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ISOLATION OF BRASSICASTEROL FROM RAPESEED OIL STEAM DISTILLATE. HENRY W. KIRCHER and FUMIKO U. ROSENSTEIN, Dept. of Agricultural Biochemistry, University of Arizona, Tucson 85721.

The sterol mixture (brassicasterol, stigmasterol, campesterol and sitosterol; ratio ~ 1:0.2:1:2) obtained by filtration of 10 gal of crude deodorizer distillate obtained from the commercial processing of rapeseed oil was crystallized, acetylated and brominated in ether-acetic acid. The ether-insoluble tetrabromides of brassicasteryl and stigmasteryl acetate were debrominated with zinc to yield 170 g of ca. 80% pure brassicasteryl acetate. After unsuccessful attempts at recrystallization of the free sterols, acetates and acetate tetra-bromides, brassicasteryl acetate was separated from stigmasteryl acetate on silver nitrate-silica gel columns with 1% ether in petroleum ether. The acetate, mp 157.5–158.5 C, was hydrolyzed to brassicasterol, mp 150–151 C, and hydrogenated over Raney nickel in ethyl acetate to 22,23-dihydrobrassicasteryl acetate in 97% yield.

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NEW NATURALLY OCCURRING COMPOUNDS FROM PEANUTS. GEORGE R. WALLER and STEVE YOUNG, Biochemistry Dept., Oklahoma State University, Stillwater 74074.

Three compounds (I–III), which gave Dragendorff positive reactions, were isolated from the basic extracts of peanut plants. Analysis of the extracts by combined gas liquid chromatography-mass spectrometry revealed that compounds I–III had molecular weights of 206, 390 and 355, respectively. Compounds II and III are present in both raw peanuts and peanut vines, whereas compound I was found only in the vine. Further analysis of partially purified preparations of compound I showed it to have an empirical formula of $C_8H_{15}N_2O_4$. Analysis of the steam distillate of peanut vines by combined gas liquid chromatography-mass spectrometry revealed the presence of 1-pentene-3-ol, 1-hexanol, linalool, α-terpineol and geraniol. None have been identified previously in peanut plants. Linalool, α-terpineol and geraniol are terpene alcohols that are common to a wide variety of plants. Preliminary evidence suggested that one of the unidentified steam volatile compounds isolated was a nitrogen-containing alcohol.

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FATTY ACID COMPOSITION OF SPANISH PEANUT OILS AS INFLUENCED BY VARIETY, SEASON, PLANTING LOCATION AND SOIL MOISTURE CONDITIONS. CLYDE T. YOUNG and RAY O. HAMMONS, Dept. of Food Science, Georgia Station, Experiment, Ga. 30212, and RALPH S. MATLOCK, GEORGE R. WALLER and ROBERT D. MORRISON.

Nine varieties or strains of Spanish botanical-type peanuts were grown in the National Variety Test in Georgia and Oklahoma, with and without supplemental irrigation for two growing seasons. The oil (of the sound mature kernels as determined by screen size) was analyzed for fatty acid composition, and the data was analyzed statistically. The data will be discussed in detail with particular emphasis on factors significantly affecting oleic (18:1) and linoleic (18:2) fatty acid composition and their ratio (O/L ratio).

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LIPID BIOCHEMISTRY AND ULTRASTRUCTURE OF DEVELOPING RAPESEED. LARS-ÅKE APPELQVIST, Dept. of Food Hygiene, Royal Veterinary College, 104 05 Stockholm, Sweden.

Quantitative changes in content and fatty acid composition of various acyl lipids have been followed in the developing seeds of a zero-erucic acid line of *Brassica napus* planted in two different growth chambers, one with an 8 hr night and the other with constant (24 hr) illumination from flowering to maturity. The seeds from plants grown under 24 hr light conditions had considerably less linolenic acid in the neutral lipids of the seeds at all comparative stages of the development. Generally also the polar lipids of these seeds had less linolenic acid than those from plants with an 8 hr dark period. The content of "membrane" lipids such as phospho- and galactolipids calculated on a per seed basis passed through a maximum during the stage of rapid accumulation of "depot" lipids, mainly triglycerides. During the later part of seed maturation, there was a very large reduction in content of galactolipids notably these rich in linolenic acid. The neutral lipids, mainly triglycerides, changed markedly in relative fatty acid pattern during seed development, but possibly with the exception of seeds sampled very late (partially germinated in the siliques?) there was no loss of neutral lipid fatty acids calculated on a per seed basis. In vitro experiments with ^{14}C-labeled malonyl CoA added to various subcellular fractions of developing *Brassica campestris* seeds have shown that the biosynthesis of the C_{20}- and C_{22}-fatty acids is particle-bound ("microsomal"). Reciprocal crossing and grafting experiments with zero-erucic and high-erucic lines of *Brassica napus* followed by fatty acid analysis of the mature seeds have also been undertaken. The results will be discussed in relation to the mode of control of erucic acid content of various tissues. Embryonic cells from rapeseeds examined at early stages of development are rich in chloroplasts of a size and structure similar to those of leaves. In later stages of development, the embryonic cells are rich in oil droplets—sometimes referred to as spherosomes—while some chloroplasts still prevail. This paper will emphasise how the changes in composition could be related to concurrent changes in the ultrastructure of the seed.

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HYDROXYLATION OF LONG CHAIN *n*-ALKANES IN THE GRASSHOPPER *Melanoplus sanguinipes*. GABY J. BLOMQUIST and LARRY L. JACKSON, Dept. of Chemistry, Montana State University, Bozeman 59715.

Secondary alcohol wax esters were recently reported by us

in the cuticular lipids of the grasshopper *Melanoplus sanguinipes*. Studies using randomly tritiated *n*-alkanes, ketones and secondary alcohols show that *n*-alkanes are hydroxylated and then esterified with saturated fatty acids. Chain length specificity is evident in the hydroxylation with C_{21} to C_{37} *n*-alkanes serving as substrates. The C_{23} *n*-alkane has the highest activity. There is less specificity evident in the esterification. Secondary alcohols from C_{18} to C_{31} are esterified.

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GAS CHROMATOGRAPHIC PROCEDURE FOR QUANTITATIVE MICROANALYSIS OF DOUBLE BOND POSITION IN HYDROGENATED OILS. E.A. EMKEN and H.J. DUTTON, Northern Regional Research Lab., ARS, USDA, 1815 N. University, Peoria, Ill. 61604.

Quantitative cleavage of epoxyoctadecanoates with periodic acid has been demonstrated and the technique incorporated into an all-gas chromatographic (GC) system for lipid analysis. The overall procedure involves three sequential GC separations interspersed by two microreactions. Samples of methyl esters were first fractionated by preparative GC and the monoenes collected and epoxidized. Next, the epoxidized samples were separated into *cis* and *trans* epoxyoctadecanoate fractions, again by GC. Then these epoxyoctadecanoate fractions were cleaved with periodic acid into aldehyde and aldehyde-ester fragments, which were analyzed by GC. The double bond positions were determined from the aldehyde and aldehyde-ester cleavage data, which were collected and processed by a computerized on-line GC data acquisition system. The procedure was tested on pure octadecenoate isomers, standard mixtures and commercially hydrogenated vegetable oils. Analyses of hydrogenated vegetable oils agreed well with data acquired by reverse-phase and argentation chromatography followed by reductive ozonolysis. The all-GC system reduced not only the total sample requirement from ca. 4 g to ca. 10 mg, but also the elapsed analysis time per sample from 3 days to 10 hr.

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DEVELOPMENT OF A QUANTITATIVE DIRTS TEST FOR COTTON LINTERS. J.W. SMITH, G.N. FERGUSON and J.D. MILLS, Buckeye Cellulose Corp.

A quantitative method for determining the dirt content of cotton linters is described. A dirt is defined as any foreign material embedded in the surface of a pulp sheet which has a marked contrasting color to the rest of the sheet. Objective data have been obtained by using a Millipore IIMC Image Analyzer to scan a semibleached handsheet that is prepared from the cooked linters product of the AOCS Official Cellulose Yield Method Bb3–47. The total area of the surface dirts is reported from this method. The precision of the method is good, and the results correlate well with the older subjective method for dirts determination. The new method has the advantage of avoiding the operator sensitive estimation of dirts and should make it possible to verify the dirts content of a given sample in different laboratories.

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ANALYSIS OF FREE OILS IN ALKYLBENZENE SULPHONATES USING HIGH SPEED LIQUID CHROMATOGRAPHY. THOMAS WOLF, SR., DYMITRI SEMIONOW and JOSEPH P. SIMKO, JR., Colgate-Palmolive Co., 909 River Rd., Piscataway. N.J. 08854.

The neutral components present in an LAS slurry are most often determined by a petroleum ether extraction. The analysis by liquid chromatography to be described shows advantages of more rapidly obtained results, improved accuracy, and differentiation between the two principal components of the free oil. The alkyl benzenes represent unreacted raw material, while the phenyl sulphones are a byproduct. It is desirable to determine these two free oil components individually. The analysis was carried out on a Du Pont Model 820 liquid chromatograph equipped with UV detection, using an adsorption column of Corasil II and mobile phase of modified heptane. At a flow rate of 3 ml/min, the two components were eluted within 5 min. Sample preparation consisted of warming with dimethyl sulphoxide, cooling and filtering. Standard deviation, four extractions, was ±0.04% for alkyl benzene (0.47% level) and ±0.01% for phenyl sulphone (0.36% level).

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URETHANE FOAMS FROM ANIMAL FATS: VII. REACTION OF EPOXIDIZED TALLOW WITH TRIMETHYLOLPROPANE AND TMP-HBr. A. BILYK, H.A. MONROE, JR., E.J. SAGGESE and A.N. WRIGLEY, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

Tallow-based polyols of higher hydroxyl content than previously obtained were prepared for use in urethane foams. Epoxidized tallow was reacted with trimethylolpropane (TMP) under catalysis by *p*-toluene sulfonic acid (2%). Reaction at 120 C in toluene was considerably more rapid than at 90 C in benzene. Hydroxyl groups were introduced by reaction of TMP both with oxirane groups and with glyceride linkages. The latter reaction conferred hydroxyl functionality even on nonepoxidized glyceride units. The hydroxyl content of polyol products increased with increase in the functional ratio of the reaction mixture, that is, the ratio of the moles of hydroxyl available from the TMP to the moles of oxirane plus ester available from the epoxidized tallow. Functional ratios of 1.4, 2.1, 4.2 and 6.6 resulted, after 10 hr reaction, in polyol products with per cent hydroxyl 6.6, 7.7, 9.5 and 10.3, respectively (compared to 4.3% OH from simple hydration of epoxidized tallow). By a modification of the process described it was possible to provide fire retardance by introduction of bromine. At the functional ratio of 6.6, epoxidized tallow in benzene or toluene was treated with TMP and gaseous HBr. Reaction at 80 C and 110 C for 12 hr gave liquid polyols of per cent hydroxyl 3.8 and 3.2; per cent bromine 30 and 42, respectively. Reaction at 80 C for 7 hr with continuous removal of water gave 7% OH and 25% Br. Examined by thin layer chromatography, the TMP-substituted polyols showed polarities in the range of mono- and diglycerides. Increase in functional ratio augmented the more polar components.

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URETHANE FOAMS FROM ANIMAL FATS: VIII. PROPERTIES OF FOAMS FROM TALLOW-TRIMETHYLOLPROPANE POLYOLS. E.J. SAGGESE, M. ZUBILLAGA, A. BILYK, G.R. RISER and A.N. WRIGLEY, Eastern Regional Research Lab., ARS, USDA, 600 E. Mermaid Lane, Philadelphia, Pa. 19118.

Polyols made by reacting trimethylolpropane (TMP) (under toluene sulfonic acid catalysis) with epoxidized tallow were converted to urethane foams by reaction with polyarylenepolyisocyanate (PAPI) in presence of triethylenediamine (Dabco), a surfactant and fluorotrichloromethane. A typical formulation was: epoxidized tallow-TMP polyol (7.7% OH), 6.5 g; triisopropanolamine, 3.5 g; Dabco, 0.25 g; silicone oil, 0.15 g; fluorotrichloromethane, 4.1 g, and PAPI, 12.3 g. The polyols, adjusted with triisopropanolamine or with an oxypropylated triamine to hydroxyl equivalent of either 100 or 120, yielded rigid foams with densities 1.5–2.0 lb/ft^3, open cell content 15–19%, and compressive strengths 34–49 psi. These values were superior to those of similar foams from hydrated epoxidized tallow. Use of only one half the normal amount of freon resulted in densities 50–70% higher, slightly lower open-cell content, and substantially higher compressive strengths. Polyols made by reaction of trimethylolpropane and hydrogen bromide with epoxidized tallow were also conveniently converted to foams. When polyol hydroxyl equivalent was adjusted to either 100 or 120 by triisopropanolamine, all foams were nonburning. At hydroxyl equivalent of 100, foams had densities 1.6–1.8, open cells 20–21% and compressive strengths 34–39 psi. At hydroxyl equivalent 120, the polyol of hydroxyl content 7.0% and bromine 25% gave foams of density 1.8, open cells 21% and compressive strength 35 psi (hydroxybrominated tallow, solvent-purified, at this hydroxyl equivalent, had previously given foams of density 1.9, open cells 19%, compressive strength 19 psi, flammability self-extinguishing). Adjustment of hydroxyl equivalent with an oxypropylated triamine gave similar physical properties but fewer foams of nonburning character. Formulation with half the normal freon gave foams of higher compressive strength but lower flame resistance.

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CONJUGATION OF POLYUNSATURATED FATS WITH DIMSYLSODIUM AND POTASSIUM. W.J. DEJARLAIS, L.E. GAST and J.C. COWAN, Northern Regional Research Lab., ARS, USDA, 1815 N. University, Peoria, Ill. 61604.

Methyl esters of polyunsaturated fatty acids were ca. 99% conjugated in 2 hr at room temperature when dimsylsodium was used, but glycerides conjugated more slowly. Dimsylpotassium is more reactive than dimsylsodium and even conjugates glycerides rapidly. Cosolvents, such as tetrahydrofuran (THF) or dialkylethers, of the various ethylene glycol polymers, are necessary with glycerides. Conjugated double bonds of methyl linoleate were about equally divided between the 9,11 and 10,12 positions. These conjugated double bonds are principally in the *cis,trans* configuration with smaller amounts of *cis,cis* isomers. Conjugated *trans,trans* isomers are formed in small amounts. From linolenate, principally conjugated diene is formed; the remaining triene is in the conjugated diene-triene form. Film properties of conjugated linseed oils were compared with those of linseed oil and alkali-conjugated linseed oil. Air-dried films containing driers and conjugated linseed oils gained weight more slowly than similar films made from untreated linseed oil. Even without driers, linseed oil films gained weight more rapidly than films containing conjugated linseed oils. Properties of baked films were also investigated.

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HYDROFORMYLATION OF METHYL OLEATE WITH A RECYCLED RHODIUM CATALYST: COST ANALYSIS FOR A BATCH PROCESS. J.P. FRIEDRICH, G.R. LIST and V.E. SOHNS, Northern Regional Research Lab., ARS, USDA, 1815 N. University, Peoria, Ill. 61604.

Methyl oleate was hydroformylated to methyl formylstearate at 120 C with 800–900 psig of a 1:1 mixture of hydrogen and carbon monoxide. An activated rhodium-on-alumina catalyst was used in the presence of triphenylphosphite. Under these conditions essentially quantitative conversion resulted in ca. 40 min. Filtration followed by distillation yielded methyl formylstearate. The solubilized rhodium catalyst was concentrated in the distillation residue. The residue was resupported on the spent support in a gas-fired rotary kiln. The process was repeated 10 times without significant loss of catalyst activity. A preliminary estimate based on a hypothetical plant producing two million pounds of product annually places the processing costs, not including cost for methyl oleate, at ca. 12 cents per pound.

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AUTOXIDATION OF POLYUNSATURATED FATTY ESTERS ADSORBED ON SILICA. JAMES F. MEAD, VIDA SLAWSON and ARTHUR W. ADAMSON, Lab. of Nuclear Medicine and Radiation Biology, Los Angeles, Calif. 90024.

It has been shown (Slawson and Mead, 1972) that the stability toward autoxidation of polyunsaturated esters is markedly increased when adsorbed on silica gel. In an extension of these studies, oxidative destruction of polyunsaturated esters, as estimated by disappearance of gas liquid chromatographic peaks relative to that of a saturated internal standard was related to silica-ester ratio, agitation, particle size and metal content of the silica. It was found that with a ratio of ester to silica considerably less than that for a monolayer, usual autoxidation kinetics are not followed, and the disappearance of ester follows a first order relationship with rates independent of the number of double bonds. The findings will be discussed from the point of view that the reaction mechanism on surfaces at these ratios is different from that at higher ratios or in solution.

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LIPID EXTRACTION TECHNIQUES AND THEIR INFLUENCE ON SUBSEQUENT FATTY ACID ANALYSIS BY GAS LIQUID CHROMATOGRAPHY AND LIPOXIDASE. A.J. SHEPPARD, A.R. PROSSER and W.D. HUBBARD, Div. of Nutrition (BF-124), BF, FDA, 200 'C' St., S.W., Washington, D.C. 20204.

Samples of corned beef hash, frozen turkey pie, frozen beef pie and beef stew were extracted by eight methods. Methyl esters of the fatty acids were prepared by the AOAC BF$_3$/methanol method and measured quantitatively by gas liquid chromatography. Total lipid extract was determined gravimetrically. Polyunsaturated fatty acids were determined as *cis,cis*-methylene interrupted polyunsaturated fatty acids by the lipoxidase method. The extraction method of choice for the

forementioned products was a 4N HCl digest followed by ethyl ether extraction. Generally, this extraction method has been effective for other products. The lone exception to date has been special dietary egg preparations. The AOAC gas liquid chromatography method had to be modified with respect to the calibration technique. The gas liquid chromatography is calibrated with pure fatty acid methyl esters; the AOAC method specifies using an oil with a fatty acid pattern closely resembling the fat product fatty acid pattern, but this proved to not be feasible for food extracts. The Canadian FA-59 lipoxidase method had to be modified to be applicable to food lipid extracts. A white precipitate interferes with the spectrophotometric readings and must be removed by centrifugation previous to obtaining final *cis,cis*-methylene interrupted polyunsaturated fatty acid absorption values in the UV range.

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COMBINED LASER PYROLYSIS GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF COMPLEX ORGANIC COMPOUNDS: I. NEUTRAL LIPIDS. E.G. PERKINS and J.C. MEANS, Burnsides Research Lab., University of Illinois, Urbana 61801.

Thermal fragmentation of organic molecules generally produces complex mixtures of compounds which are difficult to separate and identify. Laser induced pyrolysis has, in general, lead to more simplified fragmentation patterns. The mixtures resulting from the pyrolysis of lipids with a gas laser beam were subjected to gas chromatography-mass spectrometry. The fragmentation patterns resulting from the laser pyrolysis of stearic acid, methyl stearate, oxygen substituted stearate derivatives, as well as esters of glycerol, cholesterol and long chain aliphatic alcohols, were investigated. Each lipid class investigated yielded a distinctive fragmentation pattern or "fingerprint." These patterns were varied by selection of the laser control parameters of pulse duration and intensity. Examination of the mass spectra obtained from the pyrolytic fargments indicated a positive correlation between the pyrogram and the original structure of the lipid.

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THEORY AND PRACTICE OF HIGH SPEED LIQUID CHROMATOGRAPHY. R.A. HENRY and J.A. STAMM, E.I. du Pont de Nemours & Co., Inc., Instrument Products Div., Wilmington, Del. 19898.

Recent developments in high pressure pumping systems, high sensitivity detectors and efficient column packings have made liquid chromatography a technique that is rapidly taking its place with gas chromatography as a high-speed analytical tool. Unlike gas chromatography, liquid chromatography is applicable to compounds that have a low vapor pressure due to high polarity and/or high molecular weight. Compounds need only to be dissolved to be investigated. Liquid chromatography may also be used to study compounds which are too unstable for separation by gas chromatography. Because it is applicable to a greater number of compounds, many people feel that fast liquid chromatography will eventually become a more popular analytical tool than gas chromatography. Requirements of high pressure pumping systems and sensitive detectors for fast liquid chromatography will be discussed, and theoretical considerations which led to the development of efficient column packings will be reviewed. Speculation on future developments in hardware and packings will be included. A survey of some recent applications, including data on steroids, vitamins and nucleotides, will be presented, and the potential of liquid chromatography as a fast preparative tool will be discussed.

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FLAME IONIZATION DETECTORS FOR THE ANALYTICAL LIQUID CHROMATOGRAPHY OF LIPIDS. O.S. PRIVETT, W. ERDAHL and ANDRZEJ STOLYHWO, The Hormel Institute, Austin, Minn. 55912.

Application of liquid chromatography has generally been limited to those substances that can be detected by UV or refractive index-type detectors. These detectors are not widely applicable to lipids because most lipids do not have strong UV absorption properties and refractive index types do not lend themselves to gradient elution systems generally used for the separation of lipids. Hydrogen flame ionization detectors offer the most promise for the application of high speed, high resolution liquid chromatography to lipids. Detectors of this type provide universal detection of organic compounds and are highly sensitive, but their application to lipids has been only partially successful. Features and limitations of detectors of the hydrogen flame type and their associated transport mechanisms are reviewed and progress in their application to lipids described.