ABSTRACTS

R. A. REINERS, Editor. ABSTRACTORS: J. G. Endres, J. Iavicoli, K. Kitsuta, F. A. Kummerow, C. C. Litchfield, Gladys Macy, Louise R. Morrow, E. G. Perkins, and T. H. Smouse

• Fats and Oils

THE HYDROCARBONS CONTAINED IN THE UNSAPONIFIABLE FRACTION OF SOME VEGETABLE OILS. P. Capella *et al.* (Fats and Oils Exp. Sta., Milan, Italy). *Riv. Ital. Sostanze Grasse* **40**, 603–6 (1963). Vapor phase chromatography of the unsaponifiable fraction of several vegetable oils has revealed the presence of 30 to 45 hydrocarbons, plus 6 to 8 unidentified components. The unsaponifiables from linseed oil, for example, contains the complete normal paraffinic series from $C_{19}H_{22}$ to $C_{35}H_{72}$, with $C_{29}H_{60}$ in the largest proportion (8–10%), plus 11 hydrocarbons of the iso $R{-}CH(CH_3)_2$ and/or anteiso series $R{-}CH(CH_3)(C_2H_5)$, and 6–7 hydrocarbons of an unidentified series, probably olefins. It was not possible to distinguish between the iso and anteiso series, their retention times having been shown to be nearly identical. It was observed that the hydrocarbon composition is much less characteristics of the individual oils that their content in terpenic alcohols. Also, fewer hydrocarbons (29) were found in the only fruit oil examined (olive oil) than in seed oils (36–45).

ISOTHERMAL GAS CHROMATOGRAPHIC ANALYSIS OF BUTTER FATTY ACIDS. A. Daghetta and A. Jaforte (Univ. of Milan, Italy). *Riv. Ital. Sostanze Grasse* **40**, 597–602 (1963). Isothermal gas chromatography can be applied to the analysis of butter fatty acids without recourse to two chromatograms at different temperatures. The main objection to isothermal chromatography in the case of butter has been that some of the peaks are so narrow as to make the calculation of such peak areas very inaccurate. However, since peak widths are found to be proportional to distances from the origin (i.e., retention times) for all butter fatty acids, it becomes possible to calculate peak areas by means of known retention times, rather than by the more difficult to measure peak widths.

CONTRIBUTION TO THE RESEARCH OF LARD ADULTERATION. G. Jacini *et al.* (Fats and Oils Exp. Sta., Milan, Italy). *Riv. Ital. Sostanze Grasse* **40**, 584–7 (1963). The presence of tallow adulteration in lard is rather difficult to recognize with certainty by chromatographic means because of the similarity and natural variability of the fatty acids of the two materials. The authors examined the possibility of identifying tallow adulteration by observation of the dilatometric curves and the Böhmer index as well as fatty acid composition. It is claimed that, when all three methods are used, a minimum adulteration of 10% tallow in lard can be detected.

IDENTIFICATION OF ELAIDINIC ACID IN FATTY MATERIALS BY THIN LAYER CHROMATOGRAPHY. U. Pallotta and L. Matarese (Univ. of Bologna, Italy). *Riv. Ital. Sostanze Grasse* **40**, 579–83 (1963). The presence of elaidinic acid in a mixture of fatty acid methyl esters can be detected by thin layer chromatography conducted on a thin layer of silica gel mixed with silver nitrate. The separation of the elaidinic acid spot from all other C_{18} acids, as well as from acids with more or less than 18 C atoms, is very clear and the analysis itself is simple and rapid. As little as 1% elaidinic acid can be measured by this technique.

SOLUBILITY OF FATS IN N-N-DIMETHYLFORMAMIDE AND ACETIC ANHYDRIDE AS AN INDEX FOR THE IDENTIFICATION OF FATS. G. Valentinis (Hyg. Chem. Lab., Udine, Italy). *Ind. Aliment.* **2**, 81–84 (1963). The cloud points of solutions of different fats in N-N-dimethylformamide and in acetic anhydride have been determined. By using a standardized operating technique and oil:solvent ratio and provided the free acidity of the oil is first neutralized with CaO, it is possible to use the cloud point determination for the qualitative identification of the fat sample.

THE DEODORIZATION OF EDIBLE OILS. A. E. Williams. *Ind. Aliment.* **2**, 51–6 (1963). A review of current oil deodorizing technology.

THE BLEACHING OF EDIBLE OILS. *Ibid.*, 69–74. A review of currecent oil bleaching technology.

DETERMINATION OF THE FAT CONTENT OF MILK. H. E. Randolph and I. A. Gould. *Ind. Aliment.* **2**, 60–64 (1963). The Alfa-Laval method for determining the fat content of milk is described. The method is similar to the one by Babcock-Gerber, is of equal precision (±0.03%) and presents some operating advantages, such as the possibility of running a larger number of analyses per day and the fact that reading of the butyrometers can be delayed for even several days.

THE DIFFERENTIAL THERMAL ANALYSIS OF COCOA BUTTER. A Mathieu *et al. Ind. Aliment.* **2**, 57–9 (1963). Differential thermal analysis was applied to samples of cocoa butter obtained by crushing as well as by solvent extraction. A difference in the glyceride make-up of these samples is demonstrated by the presence of a characteristic peak in the cooling curve of the extracted butter that does not appear in the crushed sample. Optimum results are obtained at a cooling rate of 1.2C/min. When the samples are heated rather than cooled, no difference is found among the different cocoa butter samples.

THE EFFECT OF COMPOSITION OF COCOA BUTTER SUBSTITUTES ON THE CONSISTENCY OF CHOCOLATE COATINGS. G. Janíček, J. Pokorný and J. Kubátová (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* **6–1**, 323–37 (1962). The properties of chocolate coatings made with commercial cocoa butter substitutes cannot be easily predicted on the basis of the physical properties of the cocoa butter substitutes themselves since mixtures of cocoa butter and substitutes present different degrees of softening, depending on the type of substitute used. The intersolubility relationships between glycerides of cocoa butter and substitutes are generally so high that mixtures with only 6% substitute have considerably reduced dilation and increased penetration. In the case of substitutes containing mainly laurie and myristic acids a pronounced eutectic effect is already evident at 6% substitution, dilation of the mixture being lower than that of both components. The complete melting point of cocoa butter is not greatly affected, but the initial softening point is lowered by 4–6C, consequently such mixtures exhibit a wider melting range than pure cocoa butter. Substitutes composed predominantly of C_{16}–C_{18} fatty acids do not soften cocoa butter to the same extent as the lauric-myristic type.

SEPARATION OF LIPIDS IN THE MANUFACTURE OF SYNTHETIC SAUSAGE CASINGS FROM HIDE PARINGS. J. Pokorný and L. Ulrichová (Inst. Chem. Techno., Prague). *J. Inst. Chem. Technol. Prague* **6–1**, 207–35 (1962). Lipids constitute an undesirable component of sausage casings since they may reduce the strength and hardness of the finished product. In the process of manufacturing such synthetic casings from hide parings, most of the free lipids (petroleum ether soluble) are eliminated by saponification, while the lipids bound to the substrate (soluble only after acid hydrolysis) are not appreciably affected.

EFFECT OF PROTEIDIC SUBSTANCES FROM HIDE PARINGS ON THE OXIDATION OF FATTY ACIDS AND THEIR DERIVATIVES, I. J. Pokorný, G. Janíček and L. Ulrichová (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* **6–1**, 185–93 (1962). The presence of proteidic substances is known to have a retarding effect on the oxidation of unsaturated fatty acids, a fact which is of importance in the manufacture of edible sausage casings. Oxidative processes in this case are of two kinds: one, leading to hydroperoxides and other peroxides as well as polymers, all such compounds being completely or almost completely insoluble in common solvents. Another reaction consists in the formation of chemical bonds between oxidized fatty acids and the proteidic substrate. The products of this type of reaction are extractable with light petroleum fractions after acid hydrolysis and consist mostly of highly unsaturated fatty acids. In comparison to native lipids, added lipids uniformly distributed through the proteidic material are found to be less protected against oxidation.

EFFECT OF PROTEIDIC SUBSTANCES FROM HIDE PARINGS ON THE OXIDATION OF FATTY ACIDS AND THEIR DERIVATIVES, II. *Ibid.*, 195–205. The autoxidation reaction of fatty acids finely distributed in a proteidic substrate proceeds at a rate proportional to their degree of oxidation. The acidity of the medium does not affect reaction rate but more petroleum soluble products combined with the substrate are formed in acid than in alkaline medium. Acid conditions also favor the formation of highly polar substances, insoluble in light petroleum fractions.

EFFECT OF FAT PARTICLES ON THE MECHANICAL STRENGTH OF SYNTHETIC SAUSAGE CASINGS. L. Ulrichová and J. Pokorný

(Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–1, 237–42 (1962). The fat particles contained in synthetic sausage casings prepared from hide parings vary in size between 1 and 150 μ, the smaller particles being nearly spherical and the larger ones more noticeably elongated, as microscopically observed. It is concluded that the mechanical strength of the casings is not diminished by the presence of these fat particles, since the local fat concentration near the zones of mechanical failure is not different from the overall fat concentration (about 0.8%).

THE EFFECT OF REFINING ON THE CONSISTENCY OF HARDENED COCONUT OIL. J. Pokorný and G. Janíček (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–1, 313–22 (1962). The degree of refining accomplished on low quality coconut oil prior to its hardening does not affect the temperature range over which the hardened oil softens and melts. Deodorization, however, has been found to narrow the melting range significantly. The hardened coconut oil matches many good cocoa butter substitutes in sharpness of melting, but is only acceptable when resistance to temperatures no higher than 20C is required.

THE IODOMETRIC DETERMINATION OF PEROXIDE VALUE IN COSMETIC CREAMS, IV. J. Pokorný, I. Filípek and G. Janíček (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–1, 291–7 (1962). A modified iodometric method for the determination of peroxide value in cosmetic creams containing reducing or oxidizing substances that interfere with the common method of analysis is described. The method involves extraction of the reducing or oxidizing substances with chloroform and dilute acetic acid solution before the iodometric titration.

SEPARATION OF FATTY ACIDS FROM THEIR AQUEOUS SUSPENSIONS. A. Fuchsová and J. Zalud (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–1, 251–63 (1962). Several procedures for separating saturated and unsaturated fatty acids are described. The centrifuging of fatty acids from aqueous suspensions in solutions of surface active agents has been studied experimentally and is discussed in some detail.

LIQUID PHASE EQUILIBRIA OF SOME METHANOLIC SYSTEMS. M. Pazlar (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–1, 361–71 (1962). The liquid phase equilibria of the three systems: methanol-olive oil-oleic acid (20C), methanol-triolein-tocopherol acetate (25C) and methanol-triolein-tocopherol (25C) have been determined and empirical equations derived, correlating the composition of conjugated phases. These equations are applicable to technical calculations of extraction processes on the systems mentioned. The results on the two systems including triolein are generally valid, while the values for the third system are valid only for the particular conditions observed during the experiments.

STUDIES ON THE QUALITY ON NIGERIAN PALM KERNELS. D. G. Coursey (Federal Inst. Ind. Res., PMB 1023, Ikeja Airport, Nigeria), E. A. Simmons and (Miss) A. Sheridan. *J. West African Sci. Assoc.* 8(1), 18–28 (1963). The quality of Nigerian palm kernels, in terms of free fatty acid content, has been investigated at various stages between production and arrival in consumer countries. It has been established that substantial deterioration of quality occurs during storage. Lipolytic micro-organisms have been found to occur on palm kernels, and are probably at least partially responsible for the deterioration.

DIETHER ANALOGUES OF LECITHINS. SYNTHESIS OF DI-*O*-OCTADECYL-*L*-α-GLYCERYL-PHOSPHORYLCHOLINE. N. Z. Stanacev and E. Baer (Subdept. of Synthetic Chem. in Relation to Medical Res., Banting and Best Dept. of Medical Res., Univ. of Toronto, Toronto, Canada). *J. Biol. Chem.* 239, 410–13 (1964). The first synthesis of a *diether* analogue of a lecithin is described. The L-α-(dioctadecyl)lecithin (di-*O*-octadecyl-L-α-glycerylphosphorylcholine) was obtained by phosphorylation of D-α,β-di-*O*-octadecyl glycerol with monophenylphosphoryl dichloride and pyridine, esterification of the resulting di-*O*-octadecyl L-α-glyceryl (phenyl) phosphoryl chloride with choline iodide, conversion of di-*O*-octadecyl L-α-glyceryl(phenyl)phosphoryl choline iodide into the corresponding carbonate, and removal of the phenyl group by catalytic hydrogenolysis with platinum catalyst.

ANALYSIS OF ALCOHOLS, HYDROXY ESTERS, GLYCERIDES, AND GLYCERYL ETHERS AS NITRATES BY THIN-LAYER CHROMATOGRAPHY AND INFRARED SPECTROMETRY. D. C. Malins, J. C. Wekell, and C. R. Houle (Bureau of Commercial Fisheries Technological Laboratory, U.S. Fish and Wildlife Service, Seattle, Wash.). *Anal. Chem.* 36, 658–61 (1964). A rapid method is presented for the analysis of long-chain hydroxy compounds as their nitrate derivatives. Milligram amounts of nitrates were prepared in test tubes from fatty hydroxy compounds by reaction with acetyl nitrate. The crude reaction mixtures were then fractionated by thin layer chromatography. Because of the unique chromatographic and spectral properties of nitrates, they are more easily separated and analyzed than are the corresponding alcohols. Mono- and dinitrates were separated from other classes of compounds on thin layers of silicic acid by elution with *n*-hexane. Even weakly polar derivatives, such as esters and aldehydes, did not migrate in this system.

DIMETHOXYPROPANE INDUCED TRANSESTERIFICATION OF FATS AND OILS IN PREPARATION OF METHYL ESTERS FOR GAS CHROMATOGRAPHIC ANALYSIS. M. E. Mason and G. R. Waller (Agricultural Experiment Sta., Oklahoma State Univ., Stillwater, Okla.). *Anal. Chem.* 36, 583–86 (1964). A simple, convenient, and quantitative method for the preparation of methyl esters of fatty acids from fats and oils which does not require treatment at elevated temperatures is described. Gas chromatographic analyses are made by injecting aliquots taken directly from the reaction mixture thus eliminating evaporation and extraction steps used in other procedures. The use of 2,2-dimethoxypropane (DMP) to drive transesterification to completion eliminates the need for elevated temperatures. DMP reacts with glycerol to form isopropylidene glycerol (IPG) which serves as a convenient marker in determining retention times. This method requires only a few simple operations and is especially adaptable to routine analyses of large numbers of samples.

A PROCEDURE FOR THE SIMULTANEOUS QUANTITATIVE DETERMINATION OF GLYCEROL AND FATTY ACID CONTENTS OF FATS AND OILS. M. E. Mason, M. E. Eager, and G. R. Waller (Agricultural Experiment Sta., Oklahoma State Univ., Stillwater, Okla.). *Anal. Chem.* 36, 587–90 (1964). A procedure is described which allows the simultaneous quantitative determination of glycerol as well as fatty acids of fats and oils by gas liquid chromatography. Knowledge of the amount of both components allows a comparison of the weight of fat determined by analysis with the weight of sample used for analysis. Also, comparison of the moles of glycerol with the moles of total esters found reveals additional information concerning the purity of the fat being analyzed. Consequently, glycerol and fatty acid values are expressed on an absolute rather than relative basis.

MEAT PRESERVATION, OXIDATIVE CHANGES IN CURED AND UNCURED FROZEN COOKED PORK. M. W. Zipser, T. Kwon, and B. M. Watts (Dept. of Food and Nutr., Florida State Univ., Tallahassee, Fla.). *J. Agr. Food Chem.* 12, 105–09 (1964). A primary objective of this paper is a comparison of patterns of lipid oxidation in frozen cooked meats versus frozen cured meats. Peroxide number correlated well with sensory scores in cured samples.

CHANGES OF CHARACTERISTICS OF STARCH DURING GELATINIZATION IN THE PRESENCE OR ABSENCE OF FATTY ACID. K. Yasumatsu and S. Moritaka (Res. Laboratories, Takeda Chemical Industries, Ltd., Osaka, Japan). *J. Food Sci.* 29, 198–202 (1964). To study the effects of lipid on the gelatinization of starch, changes in its characteristics were investigated by gelatinizing the potato starch in amylograph in the presence or absence of linoleic acid. Added linoleic acid resulted in an increase in the temperature at which both viscosity and size of starch granules increased, but did not affect the temperature at which X-ray pattern and digestibility changed from those of raw starch to those of gelatinized starch.

CHEMICAL SYNTHESIS OF TRITIUM-LABELED LINOLEIC ACID. D. S. Sgoutas and F. A. Kummerow (The Burnsides Res. Laboratory, Univ. of Illinois, Urbana, Ill.). *Biochemistry* 3, 406–11 (1964). Tritium labeled linoleic acid with a specific activity of 357 mc/mmole was obtained in 70% yield from totally synthesized octadeca-9,12-diynoic acid. A source of 1 curie of tritium gas was used to semihydrogenate the diacetylenic acid in the presence of Lindlar's catalyst. Chemical and radiochemical purity of the final product were assessed by chromatographic methods. Evidence from mild oxidation in-

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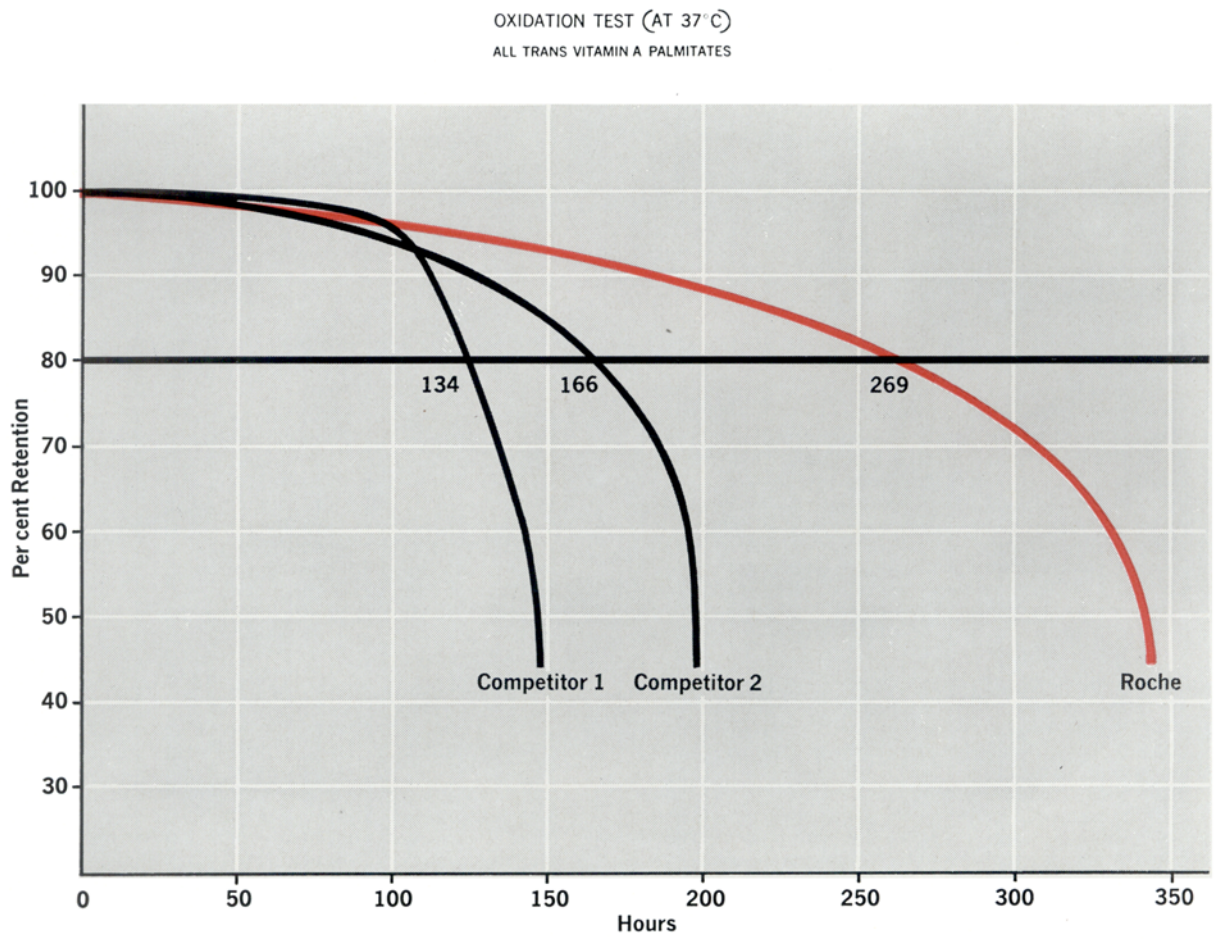

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dicated that the tritium-labeled linoleic acid was prepared with a specificity of greater than 95% of the activity at the 9, 10, 12, and 13 positions.

THE DETERMINATION AND CONTENTS OF α- AND γ-TOCOPHEROLS IN MARGARINE. G. Lambertsen, H. Myklestad, and O. R. Braekkan (Government Vitamin Lab., Norwegian Fisheries Res. Inst., Bergen, Norway). *J. Food Sci.* 29, 164–67 (1964). Methods are described for the determination of α-tocopherol and γ-tocopherol in margarine by column and paper chromatography and spectrophotometric measurement of the tocopherols. Thirty-seven samples of Norwegian margarine, average values 52 μg/g α-tocopherol and 89 μg/g γ-tocopherol, together with 18 samples from other European countries, were assayed. Ten of the samples were further assayed after 7 months of storage, and showed average losses of 20% of α-tocopherol and 14% of γ-tocopherol. Analyses carried out on 8 samples of hydrogenated fats gave tocopherol values up to the level of original oils, indicating that the hydrogenation step itself does not destroy the tocopherols. Lastly, 7 samples of mixed fat for margarine production were assayed, and gave values somewhat higher than those calculated from the margarine values, indicating some loss during margarine production.

CIS-TRANS ISOMERS OF DI-α-TOCOPHERONE. A. S. Csallany and H. H. Draper (Div. of Animal Nutr., Univ. of Illinois, Urbana, Ill.). *J. Biol. Chem.* 239, 574–77 (1964). The *cis* and *trans* isomers of di-α-tocopherone, a metabolite of *d*-α-tocopherol, have been separated and identified. The results confirm the structure assigned earlier to this compound and indicate that an alcohol-soluble form of the metabolite previously detected in liver is the *cis* isomer which was formed as an artifact of isolation.

SELECTIVE HYDROGENATION OF FATS AND FATTY OILS. F. W. Kirsch (Air Products and Chemicals, Inc.). *U.S. 3,123,626.* The method of selectively hydrogenating glyceride fats and oils in continuous operation comprises passing such glycerides through a fixed bed of solid hydrogenation catalyst at a temperature of 100–400F, a hydogen pressure of 10–150 pounds per square inch gauge and a liquid hourly space velocity of 0.1 to 4. The solid hydrogenation catalyst comprises a macroporus silica support associated with nickel and sulfur derived from nickel sulfate by hydrogen reduction. The nickel and sulfur are present in the range of 0.13 to 4.99% by weight of sulfur and 4 to 25% by weight of nickel based on the final catalyst.

FAT HYDROGENATION. W. H. Flank, J. E. McEvoy, and H. Shalit (Air Products and Chemicals, Inc.). *U.S. 3,123,627.* Fatty acid material (such as unsaturated fatty acids and esters) is heated at a reduced pressure to remove water; the vacuum dried material is then subjected to a catalytic hydrogenation zone maintained at temperatures of 50–240C. This zone contains particles of nickel on powder-containing silica gel of large pore size prepared by decomposing nickel formate dihydrate impregnated on the silica gel particles and hydrogen at a pressure from atmospheric to 4000 pounds per square inch. The molar ratio of hydrogen to fatty acid material is from 0.3:1 to 30:1; the catalyst to fatty acid material to time relationships correspond to a space rate of from 0.1 to 30 volumes of fatty acid material per volume of catalyst per hour.

METHOD OF MAKING MARGARINE LESS SPATTERING. G. M. M. Houben and E. Wilhelmus (N.V. Koninklijke Stearine Kaarsenfabrieken "Gouda-Apo'lo"). *U.S. 3,124,463.* The spattering properties of margarine are reduced by adding to the margarine up to 0.3% by weight of a partial ester product. This product is obtained by heating about 2 mols of an alcohol selected from the group consisting of alkanols having 8–16 carbon atoms, oleyl alcohol, glycerol monoethers of alkanols having 8–16 carbons and oleyl alcohol and mono-fatty acid esters of propylene glycol in which the fatty acid has 8–24 carbons with about 1 mol of citric acid at a temperature between 100 and 165C until a homogeneous mixture is obtained and then reacting another mol of citric acid with the mixture. Also added to the margarine is from 0.02% to less than 0.08% by weight of a phosphatide.

• Fatty Acid Derivatives

OPTIMUM CONDITIONS FOR THE ACETYLATION OF MONOGLYCERIDE EMULSIFIERS WITH ACETIC ANHYDRIDE. I. Tománková and J. Pokorný (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–1, 243–9 (1962). The acetylation of monoglycerides with acetic anhydride proceeds at a slow rate even under a considerable molar excess of anhydride. It is necessary to use a 450% excess of anydride to obtain 99% acetylation in 30 min reaction time. Fatty acid composition does not affect reaction rate but reaction temperature is an important factor. Reaction rate is only moderate at 100C, satisfactory at 138C (boiling point of the mixture under atm press), very good (10–15 min) at 160C, under slight pressure. The problem of losses during isolation of the product from the reaction mixture is discussed.

EMULSIFIER BLENDS AND A METHOD FOR IMPROVING FROZEN CONFECTIONS. W. H. Knightly and G. P. Lensack (Atlas Chemical Ind., Inc.). *U.S. 3,124,464.* A dry, free-flowing emulsifier composition for a frozen confection mix consists of a blend of a hard partial glyceride emulsifier having a minimum melting temperature of at least 130F and a liquid emulsifier which improves the dryness of frozen confections. The liquid emulsifier is a polyoxyethylene derivative of a higher fatty acid ester of a polyhydric alcohol containing from 3 to 6 hydroxyl groups and is present in amounts ranging from 3 to 40% by weight of the blend.

ALIPHATIC ESTERS OF UNSATURATED CARBOXYLIC ACIDS. S. Altscher and T. F. Groll, Jr. (Nopco Chemical Co.). *U.S. 3,124,602.* A process for preparing a nonionic, water-soluble material comprises the steps of condensing from about 1 mole of glycerine and from 15–27 moles of ethylene oxide in the presence of a condensation catalyst and then esterifying from 1.3 to 2.15 parts by weight of the resulting glycerine-ethylene oxide condensate with about 1 part by weight of a carboxylic acid selected from the group consisting of oleic, linoleic, linolenic and abietic acids and mixtures thereof.

• Biology and Nutrition

BIOSYNTHETIC FAT PRODUCTION BY RHODOTORULA GRACILIS IN SULPHITE WASTES. J. Dyr and J. Protiva (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–1, 125–34 (1962). Experiments have been conducted on the cultivation of the yeast *Rhodotorula Gracillis* in sulphite wastes, with small addition of molasses. Optimum results gave a yield of about 2% yeast solids, with a content of 62% fat, having a saponification value of 192 and an iodine value of 78.

VITAMIN A TOXICITY AND ITS EFFECT ON LIVER NUCLEIC ACIDS, PROTEIN AND LIPIDS IN THE CHICK. R. L. Squibb (Lab of Disease and Environmental Stress, Rutgers, The State Univ., New Brunswick, N. J.). *Poultry Sci.* 42, 1332–35 (1963). White Leghorn cockerels 1 to 3 weeks of age were injected daily for 13- and 23-day periods with 1 ml of cottonseed oil containing 150, 25,000, 50,000 and 100,000 IU's of vitamin A palmitate. Depressed body weight feed utilization, together with increased mortality, indicated the 100,000 IU level to be toxic. Further, at this level of vitamin A, there was a decrease in liver protein and an increase in lipids. The depressing effect of high levels of vitamin A on cellular protein is discussed.

EFFECT OF VITAMIN A, VITAMIN E AND ETHOXYQUIN ON THE REPRODUCTIVE PERFORMANCE OF TURKEYS. R. L. Atkinson, A. A. Swanson, J. R. Couch and J. H. Quisenberry (Dept. of Poultry Sci., Texas A and M Univ., College Station, Texas). *Poultry Sci.* 42, 1380–86 (1963). Turkey hens fed a soybean meal and milo ration, containing all known nutrients with the exception of vitamin A, stopped laying after approximately 50 days and exhibited typical vitamin A- deficiency symptoms unless the ration also contained ethoxyquin. Vitamin E was not effective in preventing these symptoms. The addition of vitamin E to the basal ration gave normal hatchability. Ethoxyquin gave good hatchability but at a somewhat lower level than vitamin E.

EFFECT OF HIGH LINOLEIC ACID AND ANTIOXIDANT DEFICIENT DIET ON BLOOD SERUM PROTEINS IN CHICKS. L. L. Tureen, J. Conomy

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and D. K. Lee (Max and Anna Tureen Neurology Res. Lab., and Dept. of Neurology and Psychiatry, St. Louis Univ. School of Med., St. Louis, Mo.). *Proc. Soc. Exp. Biol. Med.* **115**, 429–33 (1964). Chicks fed on an experimental diet, high in linoleic acid and deficient in antioxidants, developed a progressive disease manifested by integumentary changes and signs of central nervous system impairment. Blood serum protein patterns were altered and were characterized by an increase in total protein, a decrease in the A/G ratio, and increase in beta and alpha 2 globulin fractions. Despite a decreasing A/G ratio, chicks on the experimental diet did not develop exudative diathesis. It appears that the blood serum protein changes are a manifestation of vitamin E deficiency.

EFFECT OF CERTAIN COMPOUNDS ON SOLUBILITY OF CHOLESTEROL IN COCONUT OIL. L. D. Wright and J. A. Presberg (Graduate School of Nutr., Cornell Univ., Ithaca, N. Y.). *Proc. Soc. Exp. Biol. Med.* **115**, 497–504 (1964). A simple assay method has been developed for determining the effect of various compounds on the solubility of cholesterol in oils. The method depends on the counting of an aliquot of supernatant solution following the equilibration of oil, an excess of cholesterol-4-C^{14}, and compound studied with respect to influence on the solubility of cholesterol. It was confirmed that the solubility of cholesterol in coconut oil is decreased by the presence of α-sitosterol. The effect is specific as far as a large number of sterols is concerned.

THE FATTY ACID COMPOSITION OF LIVER LIPIDS FROM RATS RAISED ON PORK RATIONS. I. J. Tinsley (Dept. of Agricultural Chem., Oregon State Univ., Corvallis, Ore.). *J. Food Sci.* **29**, 130–35 (1964). The liver lipid fatty acid composition of animals raised on pork rations was determined and compared with that of animals raised on a stock ration. The pork rations contained approximately 25% crude lipid, the proportion of oleic acid being 46–50%. The relation between the dietary and liver lipid fatty acid compositions was evaluated, with the most striking relationship being that between dietary and liver lipid oleate/linoleate ratios. The tissue levels of oleic, linoleic, and arachidonic acids provided supporting evidence for the existence of a competitive effect of oleic acid in the conversion of linoleic to arachidonic acid. The results suggest that the dietary oleate/linoleate ratio is of importance in essential fatty acid nutrition in rations containing appreciable quantities of oleic acid. The sex variable, as it applies to the relation between dietary and liver lipid, was also evaluated.

EFFECT OF STRESS FROM HIGH PROTEIN DIETS ON VITAMIN A METABOLISM IN CHICKS. G. S. Stoewsand and M. L. Scott (Dept. of Poultry Husbandry and Graduate School of Nutr., Cornell Univ., Ithaca, N.Y.). *J. Nutr.* **82**, 188–96 (1964). High protein diets fed to young chicks for 4 weeks produced a stress as evidenced by hypertrophy, hyperactivity and depletion of phospholipid content of the adrenal cortex and at the same time, caused an increased demand on vitamin A nutrition. No adrenal hypertrophy or hyperactivity occurred in 4-week old chicks fed high protein diets and simultaneously injected daily with corticosterone. However, daily injections of corticosterone increased the level of vitamin A in the blood plasma and generally caused a decrease in liver vitamin A levels. Although vitamin A deficiency was not found to affect adrenal size or corticoid production, the *in vitro* addition of vitamin A to incubated adrenals from chicks fed high protein diets caused a marked increase in corticosterone production.

HYPERTENSION AND CARDIOVASCULAR ABNORMALITIES IN STARVED-REFED SWINE. G. S. Smith, J. L. Smith, M. S. Mameesh, J. Simon and B. C. Johnson (Dept. of Animal Sci. and Dept. of Veterinary Pathology, Univ. of Illinois, Urbana, Ill.). *J. Nutr.* **82**, 173–82 (1964). Heart rate and blood pressure were recorded daily in 2 groups of young adult swine during many months of experimentation involving repeated episodes of starvation (total feed deprivation) and refeeding. Electrocardiograms from the standard and augmented limb leads were recorded during various phases of the study. Severe stresses upon the cardiovascular system were produced as a result of unrestricted initial refeeding particularly, with glucose alone or with a diet high in glucose. Damage of apparently irreversible nature was produced in the myocardium, arteries, and arterioles as a result of the stresses in refeeding following starvation. Notable diastolic hypertension was evident after only 2 starvation-refeeding episodes, and persisted following the fourth episode until the animals were killed 4 to 6 months later.

METABOLISM OF ISOLATED FAT CELLS. I. EFFECTS OF HORMONES ON GLUCOSE METABOLISM AND LIPOLYSIS. M. Rodbell (Lab. Nutr. and Endocrinology, Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.). *J. Biol. Chem.* **239**, 375–80 (1964). Studies were made on the metabolism of isolated fat cells and stromal-vascular cells prepared by collagenase treatment of rat epididymal adipose tissue. The isolated fat cells metabolized glucose by the same pathways as intact adipose tissue and accounted for essentially all of the glucose metabolism observed in this tissue. Free fat cells also maintained the different metabolic characteristics observed in adipose tissue from fasting and fed rats. It is concluded that isolated fat cells retain the ability to metabolize both glucose and triglycerides and respond to several hormones that have been shown to affect the metabolism of adipose tissue.

EFFECT OF MONOGLYCERIDES ON ABSORPTION OF CHOLESTEROL FROM THE INTESTINE AND TURNOVER RATE OF CHOLESTEROL ESTERS IN PLASMA AND LIVER OF THE RAT. K. G. Pinter, O. N. Miller and J. G. Hamilton (Dept. of Biochem., and Nutr. and Metabolism Res. Lab., Dept. of Med., Tulane Univ. School of Med., New Orleans, La.). *Proc. Soc. Exp Biol. Med.* **115**, 318–23 (1964). Absorption and transport of cholesterol were studied in rats with gastric and venous cannula. These studies were made on individual experimental animals by periodically sampling the blood of the same animal. Absorption of the cholesterol from the intestine depends upon the degree of saturation of the dietary fat available in the intestine. More cholesterol is absorbed when polyunsaturated fat is present than when saturated fat is present in the intestinal lumen.

EFFECT OF A FAT-FREE MATERNAL DIET ON THE FATTY ACID COMPOSITION OF THE PROGENY. H. Menge, E. C. Miller and C. A. Denton (Poultry Res. Branch, Animal Husbandry Res. Div., ARS, USDA Agricultural Res. Center, Beltsville, Md.). *Poultry Sci.* **43**, 164–68 (1964). Essential fatty acid depletion of dams resulted in a reduction of the level of polyunsaturated fatty acids and an increase in monounsaturated fatty acids in the plasma and heart fat of day-old progeny. During the depletion period, the linoleic acid level decreased more rapidly than the arachidonic acid level in the heart and plasma fat of the progeny. The major differences in the fatty acid composition of fat-deficient progeny (F-F) and progeny of hens receiving fat

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with. The unesterified acids are difficult to gas chromatograph, because of their tendency to dimerize and adsorb irreversibly on the columns. James and Martin also discovered some of the best liquid phases for separating the higher fatty acid esters; namely, the Apiezon greases. These researchers then embarked on a program to study the biological lipid systems. For the first time, scientists had available a simple tool for doing this. Up to this time, the methodology was slow, cumbersome, generally inaccurate, and lacked resolution. This pioneer work set a pattern in GLC fat analysis that is still followed today.

The Apiezon columns gave good separations of fatty acids by chain lengths and separated methyl stearate and methyl oleate well. However, these columns had several drawbacks. It took several hours for methyl stearate to emerge from a high efficiency column. (A short time earlier, the analysis could not be done, so now we were unhappy because it took two hours). The Apiezon columns also would not separate the polyunsaturated acids from each other.

In 1957 the National Heart Institute, inspired by the current fat and heart disease relation theories, formed the Lipid Analysis Committee (LAC). The purpose of this group was to find better analytical methodology for studying lipid metabolism. One of the first problems was to find a liquid phase that would overcome the drawbacks of the Apiezon greases. Several members of the committee, Orr, Callen, and Lipsky, came up with the polyester liquid phases. Columns utilizing these liquids would separate the methyl esters through C-18 in less than 30 min. Furthermore, they could separate the saturated esters from the unsaturated esters with astounding ease. The best phases were diethylene glycol-succinic acid polyester, ethylene glycol-adipid acid polyester, and many similar polymers. Now was the time for everyone to start getting into the act!

Gas Chromatography in Process Control

With the advent of the polyester liquid phases, routine analyses of fatty acids in fats and other lipid materials became possible. In less than a decade, gas chromatography advanced from a laboratory curiosity to a highly sophisticated analytical instrumental technique.

In industry, the analysis of the fatty acids by GLC has found very wide application. The characterization of fatty acids in triglycerides, oils, drying oils, and polyesters, are some of the most common applications. The fatty acid

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Gas Chromatography in Research

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composition of many fats and oils became the subject of a great number of papers. Almost all the tables of fatty acid composition of lipids have become subject to revision. Considering the tremendous number of sources of fatty materials, this is a formidable task. This highly important work will undoubtedly take many years to compile and compile and complete.

Many of us were told in school that natural occurring fatty acids came only in even numbered carbon chain lengths. It was not long before the gas chromatographers found an amazing variety of "odd-ball" acids to complicate our lives. These include normal odd numbered, branched odd and even number, iso, and anteiso acids. Of course, these acids occur in small amounts, but they are of great biochemical interest. The biochemists have made great use of GLC in their field. They have studied the lipids in the arterial wall, in brain tissue, the sebum of humans and animals, to name a few applications. The biomedical researchers have made studies of blood and tissue lipids and their relation to various diseases. This area of reseach in just really beginning and has a promising future for diagnostic studies.

Just about every fatty acid one can think of has been gas chromatographed, usually as its methyl ester or as some other volatile derivative. There occur in nature a tremendous variety of fatty acids which had made the analysis of fats a very difficult problem. Gas chromatography has been a great step toward solving this problem. Particularly with those fats that contain minor small amounts (0.5% or less) of the unusual fatty acids. Fats having 40 or more fatty acid components are not unusual. Without the powerful separating ability of gas chromatography, analyses of these fats would be all but impossible. Like many great technical achievements, gas chromatography has unintentionally complicated our lives with all the data it makes available.

The application of gas chromatography to the fat and oil field has not been restricted to fatty acids and their esters. One of the more ambitious applications is the direct analysis of mono-, di- and triglycerides. With all the combinations the fatty acids can form with glycerine, an oil such as cocoanut oil must be extremely complicated. Nevertheless, the gas chromatography of some fats has been done using temperature programming and short columns using a silicone gum rubber liquid phase. There is no doubt that this work will be improved and will lead to a greater knowledge of the structure of fats and oils than has ever been achieved before. This information could lead to the making of tailormade fats and have a great affect on future studies of lipid metabolism and fat nutrition. Those of us who

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have been involved in analytical work and quality control for many years have had nightmares contemplating the probable future. The specifications for a future fat may be something like this: 12:12:12 (trilaurin), 14:14:14 (trimyristin), 16:16:16 (tripalmitin), and 18:18:18 (tristearin) in equal quantities. You won't be able to hedge because the customer will also have a gas chromatograph. Someone once said gas chromatography will make honest men out of us all.

Fatty acids are the starting material for a great many derivatives. These include long chain alcohols, aldehydes, nitriles, amines, ketones, amides, and quaternary ammonium compounds. Gas chromatography has been applied to the separation and analysis of each of these materials. Before GLC, it was necessary to develop a functional group analytical method for each new compound as they were made. Very often, more time was required to find a suitable analytical method than it took to develop the original compound. It goes without saying, that an enormous amount of time is being saved in research projects through the use of GLC. Another dividend, for the analytical chemist at least, is the ease of convincing the organic chemists and other researchers their compounds are not always pure. When one observes a dozen peaks in a chromatograph of a "pure" compound, its purity becomes suspect.

Up to now we have only discussed direct GLC applications in the lipid field. There are many other analytical applications for GLC of interest to the fat and oil industry. These applications include investigations on pesticides in fats, chick edema factor, flavors, odor and color problems. The great sensitivity of GLC will probably make the solution of these problems possible or at least somewhat easier.

Gas chromatography complements many other analytical methods. Used in combination with other instrumental techniques, the scope of each method is increased. The effluents from the GLC column can be further examined using IR and UV absorption spectrometers. Another very useful technique is to pass part of the gas stream directly into a mass spectrometer. Radioactive fatty acids and esters have been chromatographed and their radioactivity measured as they emerge from the column. The effluents of the gas stream may be passed into chemical reagents to indicate functional groups. Another common practice is to collect the peaks for further characterization by chemical and physical means. Many devices have been invented for this purpose. Some are as simple as a glass capillary in a paper cup of dry ice. Others are complex electronically controlled fraction collectors capable of automatically collecting up to 50 peaks. Once a peak is collected, a melting point may be run on it or perhaps IR or UV spectroscopy analysis. If the collected peak is radioactive, its radioactivity may be measured with a liquid scintillation counter. From all this, one can surmise rightly that it often takes more than a retention time to prove the identity of an unknown gas chromatographic peak.

Another useful technique that complements GLC is thin layer chromatography (TLC). Spots from thin layer chromatographs can be recovered and gas chromatographs may be collected and rechromatographed on thin layer plates for proof of purity and identity. It should be mentioned that the older techniques of column and paper chromatography can also be used in much the same way as TLC to complement GLC. However, these earlier methods cannot match the resolving power or ease of operation of either GLC or TLC.

Since the earliest days of gas chromatography, it has always been possible to collect enough material from an analytical chromatograph for further analytical work. Investigators soon visualized scaling up GLC to obtain significant quantities of very pure substances. At first these early preparative instruments were nothing more than scaled up plumbing. Columns of one to ten inches in diameter were often constructed. It was quickly found that though these large columns had great capacity, their efficiency was usually very poor. The modern approach is to use smaller diameter columns with automatic injection and collection. Such an instrument may be programmed to run continuously until considerable quantities of the desired pure substances are collected. A number of such instruments are available commercially. Unfortunately other factors can complicate preparative GLC. These are column bleed, enrichment of trace impurities, and contamination with late appearing peaks in the automated instruments. It may be worthwhile commenting on some of these effects. When we first began to use automated preparative gas chromatography on fatty materials, we collected the desired peak over and over from many sample injections until a considerable quantity was obtained. Analytical gas chromatography was then used to assay the material. It was with considerable dismay that we found we had not the single *pure* compound as we expected. We had enriched some trace materials that emerged very close to the main peak and had an obvious mixture. Compounds that were normally only in trace amounts, were now present in appreciable quantities. This trace enrichment could be used to advantage to obtain the compounds, but it can complicate the purification process.

The above mentioned problem has another variation in that materials with much longer retention time can contaminate the peaks being collected. This happens with instruments that program through a preset time cycle. If

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in their diet (L-S) existed in the levels of arachidonic, linoleic, and a C-20 triene. The F-F chicks, which were late in hatching, had a lower level of linoleic and arachidonic and a higher level of a C-20 triene than L-S chicks that hatched normally. The length of time the dams were depleted of essential fatty acids had no effect on the fatty acid composition of the tissue fat of progeny fed a practical diet for 4 weeks.

BIOSYNTHESIS OF UNSATURATED FATTY ACIDS IN ACANTHAMOEBA SP. E. D. Korn (Lab. of Biochem., Section of Cellular Physiol., Natl. Heart Inst., Nalt. Insts. of Health, Bethesda, Md.). *J. Biol. Chem.* 239, 396–400 (1964). The biosynthesis of the 18 carbon and 20 carbon unsaturated fatty acids of *Acanthamoeba sp.* has been studied by growing the amebas in the presence of radioactive fatty acids. Evidence has been obtained for the pathway acetate → stearate → oleate → linoleate → 11,14-eicosadienoate → 8,11,14-eicosatrienoate → 5,8,11,14-eicosatetranoate. Oleate is also converted to 11-eicosenoate.

STUDIES ON AVIAN FAT COMPOSITION. 1. EFFECT OF DIETARY FAT ON THE FATTY ACIDS OF THE TRIGLYCERIDES AND PHOSPHOLIPID FRACTIONS OF THE BLOOD PLASMA AND ADIPOSE TISSUE LIPIDS OF THE LAYING HEN. R. E. Isaacks, R. E. Davies, T. M. Ferguson, R. Reiser and J. R. Couch (Dept. of Poultry Sci. and Biochemistry and Nutrition, Texas A&M Univ., College Station, Texas). *Poultry Sci.* 43, 105–13 (1964). An attempt has been made to study the relationship between the fatty acid composition of the blood plasma and adipose tissue of two year old hens that had been maintained on four different rations, three of which contained either 10% animal tallow, 10% rice oil, or 10% soybean oil. Both the triglyceride and phospholipid fraction of the blood plasma and adipose tissue were analyzed. The substitution of fat in the diet, either animal or vegetable, for rice hulls resulted in an increase in the total per cent unsaturated acids and a decrease in the saturated acids of the plasma triglycerides and plasma phospholipids, but had no effect on the saturated to unsaturated acid ration of the adipose phospholipid.

2. THE SELECTIVE UTILIZATION OF FATTY ACIDS BY THE CHICK EMBRYO. *Ibid.*, 113–20. The polyunsaturated acids of the egg yolk triglyceride and phospholipid appear to be perferentially utilized in the development of the 20-day chick embryo. The saturated acids, myristic acid, palmitic acid, and stearic acid increased in concentration in both the remaining yolk triglyceride and phospholipid fractions. A decrease in per cent of oleic acid in the remaining yolk sac triglyceride fraction indicates its preferential utilization, while a decrease in per cent of linoleic acid in the remaining yolk sac phospholipid fraction also suggests preferential utilization. Analysis of the 20-day embryo triglyceride and phospholipid fraction shows the synthesis of a more saturated fat in both fractions than occurred in the triglyceride fraction or phospholipid fraction of the egg yolk lipids.

PHOSPHOLIPID-SUGAR COMPLEXES IN RELATION TO CELL MEMBRANE MONOSACCHARIDE TRANSPORT. P. G. LeFevre, K. I. Habich, H. S. Hess, M. R. Hudson (Dept. of Pharmacology, Univ. of Louisville School of Medicine, Louisville, Ky.). *Science* 143, 955–57 (1964). Phospholipids extracted from "ghosts" of human erythrocytes or from other sources carry substantial quantities of glucose or other monosaccharides from the dry state into highly nonpolar solvents. Various characteristics of this weak association phenomenon show suggestive parallels with known properties of the mediated sugar-transfer system in the membrane of the intact red cell.

PROTEIN-ENERGY RELATIONSHIP IN ADULT RATS. II. QUANTITATIVE VARIATIONS IN DIETARY NITROGEN AND EFFECTS ON DEPOSITION OF HEPATIC FAT AND ON NITROGEN UTILIZATION. P. A. Garcia and C. E. Roderuck (Home Economics Research Dept., Iowa Agricultural and Home Economics Experiment Sta., Ames, Iowa). *J. Nutr.* 82, 231–36 (1964). Groups of female adult rats were fed, *ad libitum*, a diet providing 5% of the food energy as lactalbumin or the same diet supplemented with amino acids calculated to the most limiting after comparison of the ratio of amino acids to tryptophan with that of the daily amino acid requirements of the adult rat. After 20 days of unrestricted feeding, no significant differences in food intake were attributable to added arginine, methionine, phenylalanine and valine. Both groups were comparable in body weight, nitrogen retention and in weights and nitrogen concentrations of liver and carcass. However, hepatic fat was significantly lower with supplementation than without. Restriction of food energy intake for 30 days to two-thirds of amounts consumed voluntarily was associated with decreased hepatic weight and nitrogen; concentration of hepatic nitrogen increased.

LIPIDS OF THE EMBRYONIC LIVER. G. L. Feldman and C. K. Grantham (Biochem. Sec., Dept. of Ophthalmology, Baylor Univ. College of Med., Houston, Texas). *Poultry Sci.* 43, 150–53 (1964). The accumulation of lipids in the embryonic liver increases very rapidly after the seventeenth day of incubation. Most of this increase is the result of a marked build-up of cholesterol ester which may play a role in enabling the chick to adapt to its new environment after hatching. The liver lipids are all similar in fatty acid composition, differing only in the quantities of palmitate, stearate and oleate that occur.

EFFECT OF RESTRICTED ACCESS TO FEED ON THE SERUM AND LIVER LIPIDS OF SWINE. H. D. Fausch (Animal Sci. Dept., California State Polytechnic College, Pomona, Calif.). *Proc. Soc. Exp. Biol. Med.* 115, 402–05 (1964). Serum cholesterol and phosphoslipid levels were significantly elevated ($P < 0.05$) in *ad libitum*-fed pigs compared with the restricted-fed animals at the end of 84 days. After 173 days of restricted feeding, pigs fed *ad libitum* had significantly higher liver cholesterol, triglyceride and C/P ratios than pigs with limited access to food. The serum C/P ratio was significantly higher but serum triglyceride significantly lower ($P < 0.05$) in the *ad libitum*-fed pigs when compared with the group receiving one 2-hour feeding. Marked variations in the serum lipid levels between different breeds of pigs and between sexes were observed.

PEROXIDATION AND LYSOSOMES IN NUTRITIONAL MUSCULAR DYSTROPHY OF CHICKS. I. D. Desai, C. C. Calvert, M. L. Scott and A. L. Tappel (Dept. of Poultry Husbandry and Graduate School of Nutr., Cornell Univ., Ithaca, N.Y., and Dept. of Food Sci. and Tech., Univ. of California, Davis, Calif.). *Proc. Soc. Exp. Biol. Med* 115, 462–66 (1964). Muscle tissues from chicks with nutritional muscular dystrophy produced by feeding a diet deficient in Vitamin E and sulfur amino acids and stressed with excess dietary linoleic acid showed significant increases in peroxidation as evidenced by TBA index and also showed increased activities of the lysosomal enzymes. Addition of DL-methionine or d-α-tocopheryl acetate to the deficient diet prevented the dystrophy, reduced the peroxidation and reduced the lysosomal enzyme activities.

LIPID COMPOSITION AND SYNTHESIS IN RAT LIVER DURING PREGNANCY AND THE PUERPERIUM. W. H. Dannenburg, R. L. Burt and N. H. Leake (Dept. of Obstetrics and Gynecology, Wake Forest College, and North Carolina Baptist Hosp., Winston-Salem, N.C.). *Proc. Soc. Exp. Biol. Med.* 115, 504–08 (1964). The composition and incorporation of acetate-1-C^{14} into liver lipids of non-pregnant, pregnant, and puerperal rats were determined. Results show that total cholesterol and phospholipid phosphorus in rat liver during pregnancy and the puerperium do not differ from those found in non-pregnant animals. However, the triglycerides from livers of puerperal animals were significantly higher than those found in ether non-pregnant or pregnant rats. Incorporation of acetate-1-C^{14} into fatty acids and unsaponifiable substances by liver slices from pregnant rats was significantly greater than that observed for non-pregnant and puerperal rats. No differences were observed in the μmoles of acetate incorporated into the fatty acids of triglycerides and phospholipids.

METABOLISM AND EXCRETION AS FACTORS INFLUENCING SERUM CHOLESTEROL LEVEL IN TWO LINES OF CHICKENS. C. E. Clark, F. H. Wilcox and C. S. Shaffner (Dept. of Poultry Sci., Univ. of Maryland, College Park, Md.). *Proc. Soc. Exp. Biol. Med.* 115, 524–27 (1964). Two lines of White Leghorn chickens differing in serum cholesterol level were studied. The line difference in cholesterol was unchanged in serum from post-hepatic blood as compared to pre-hepatic blood. No line differences were observed in rate of *in vitro* cholesterogenesis from acetate-1-C^{14} incubated with blood or liver slices. Following administration of cholesterol-4-C^{14}, differences were not detectable between lines in labeled nonsaponifiable substances in liver, gut, skin, adrenal, or carcass, or in the labeled saponifiable fraction of liver or feces. Fecal excretion of labeled nonsaponifiable substances, however, was significantly higher in the low line, suggesting genetic regulation of cholesterol homeostasis through excretory pathways.

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INFLUENCES OF DIETARY CARBOHYDRATE-FAT COMBINATIONS ON VARIOUS FUNCTIONS ASSOCIATED WITH GLYCOLYSIS AND LIPOGENESIS IN RATS. II. GLUCOSE VS. SUCROSE WITH CORN OIL AND TWO HYDROGENATED OILS. C. Carroll (Dept. of Home Economics, Univ. of Arkansas, Fayetteville, Ark.). *J. Nutr.* **82**, 163–72 (1964). Weanling rats were fed diets differing only in source of carbohydrate and fat for 2 to 4 weeks. Livers were assayed for glucose-6-phosphatase and fructose diphosphatase activities, and for content of glycogen and lipids. Effects on enzyme activities of substituting fructose for glucose were similar to those observed on substituting sucrose for rice starch. Feeding either hydrogenated coconut oil (HCO) or hydrogenated peanut oil (HPO) in place of corn oil (CO) modified the enzymatic responses to dietary fructose. Results with HPO were somewhat different than those with HCO. Labile phosphorus values were highest in groups fed sucrose or fructose with CO, and lowest in those fed rice starch or glucose with HPO.

PURIFICATION AND CHARACTERIZATION OF THE LIPID A COMPONENT OF THE LIPOPOLYSACCHARIDES FROM ESCHERICHIA COLI. A. J. Burton and H. E. Carter (Division of Biochem., Noyes Lab. of Chemistry, Univ. of Illinois, Urbana, Ill.). *Biochemistry* **3**, 411–18 (1964). A crude lipopolysaccharide fraction has been isolated from *Escherichia coli*, strain $O_{111}B_4$, by cold aqueous phenol extraction. Purification of this material gave a lipopolysaccharide preparation (F-III) of high molecular weight in 4–5% yield (based on the dry weight of the cells). In addition, by extending the purification procedures commonly used, another 10–15% yield of lipopolysaccharide was recovered. The lipid content of this second preparation (F-IV) has not been previously observed. Mild aqueous acid degradation of the bacterial lipopolysaccharides released the Lipid A component, which was then purified by acetone fractionation and silicic acid chromatography.

PURIFICATION PROCESS OF TOCOPHEROL CONTAINING MATERIALS. S. Kijima, K. Naito and T. Mori (Eisai Co., Ltd., Tokyo). *U.S. 3,122,565.* A tocopherol containing material is dissolved in a polar organic solvent and contacted with a basic anion exchange resin in hydroxyl form to adsorb substantially all of the tocopherols on the resin. The adsorbed tocopherols are eluted by passing an acidic eluting solution through the resin.

STABLE DRY POWDERED FAT-SOLUBLE VITAMINS. A. Rosenberg (Commercial Solvents Corp.). *U.S. 3,124,510.* A method of manufacturing dry, powdered, fat-soluble vitamins in a stable form comprises mixing water, fat-soluble vitamin-bearing oil having a high concentration of the vitamin, edible fat which is a normally solid edible fat having a melting point ranging from 35–75C and a non-fat vehicular material comprising a combination of gelatin and skim milk solids, homogenizing the mixture, and spray-drying the homogenizate.

CAROTENOID PIGMENT AND PROTEIN COMPLEX AND METHOD OF PRODUCING THE SAME. W. H. Wingerd and S. Saperstein (Borden Co.). *U.S. 3,125,451.* In making a carotenoid composition of improved color retention, the process comprises admixing 0.005–10 parts by weight of a carotenoid and 40–300 parts of an edible lipid solvent into an aqueous dispersion, at a temperature of at least 160F, of 100 parts of a protein (milk, soy or peanut protein) and that is soluble in water only at a pH of 5–9, homogenizing the resulting mixture, maintaining the mixture at a pH within the range 5–9 and below the temperature of heat denaturing of the protein until a complex of the carotenoid and the protein results. A substantial part of the carotenoid thus becomes unextractable from the protein by petroleum ether.

• Drying Oils and Paints

DRYING OILS: ISANO OIL. J. D. von Mikusch. *Farbe Lack* **70**, 17–28 (1964). If one disregrads linseed and tall oil, there is a distinct lack of new and comprehensive information on the individual drying oils which can claim to be of interest in the manufacture of binders. This is the first of a series of papers which will deal with drying oils. The author has given a complete picture of this history, isolation and uses of isano oil.

ELECTRO-DEPOSITION OF PAINT—1 AND 2. A BEGINNER'S GUIDE TO "ELECTROPHORESIS." J. R. Berry. *Paint Technol.* **27**, 13–18 (1963); **28**, 24–28 (1964). An excellent introduction to the technology.

DRYING PROPERTIES OF THE SEED OIL FROM SIBERIAN PINE. J. Pokorný and V. Pokorná (Inst. Chem. Technol. Prague). *J. Inst. Chem. Technol. Prague* **6–1**, 285–90 (1962). The oil extracted from the seeds of the Siberian Pine contains a high percentage of linoleic and a moderate amount of linolenic. Films prepared from this oil dry more slowly and are less hard than films prepared from common drying oils. However, addition of up to 50% of this pine seed oil to linseed oil or tung oil does not cause a substantial drop in the drying rate of the latter oils. Drying is accelerated by the addition of promoters, especially manganous and cobaltous salts.

• Detergents

HYDROTROPES: SOLUBILISING AGENTS FOR DETERGENT POWDERS. MANUFACTURE OF SODIUM P-TOLUENE SULPHATE. M. A. Phillips. *Mfg. Chemist* **34**, 575–77 (1963). An economic study of the commercial production of sodium *p*-toluene sulphate is presented.

THE EFFECT OF TEMPERATURE ON THE SULPHATION OF CONDENSATION PRODUCTS OF DI- AND TRIETHANOLAMINE WITH OLEIC ACID. J. Zajíc and J. Kahoveová (Inst. Chem. Tech., Prague). *J. Inst. Chem. Technol. Prague* **6–1**, 265–83 (1962). Diethanolamine and triethanolamine were reacted with oleic acid (200 mm Hg, 160C, 0.5% sulfuric acid catalyst) and the reaction products analyzed as 60% amide and 40% esteramine in the case of diethanolamine, 100% esteramine in the case of triethanolamine. These condensation products were sulphated with cone H_2SO_4 (up to a 3:1 molar excess) at temperatures from 0–80C. The maximum degree of double bond sulphation was found to be approximately 60% under optimum conditions, i.e. 40–60C and 100% excess H_2SO_4. Surface tension of a 3 g/l solution of the sulphated products was 45–55 dyn/cm, detergency only 50% as high as that of alkylbenzene sulfonate but foam stability (Ross-Miles method) is very high (93%).

ROLE OF EXTERNAL VARIABLES IN DEGRADATION OF STRAIGHT CHAIN ABS. R. Fuhrmann, J. van Peppen and W. Ford (Allied Chemical Corp.). *Soap Chem. Specialties* **40**(2), 51–53, 106 (1964). The influence of the following external variables on the adaptation and rate of degradation of straight chain alkyl benzene sulfonates is discussed: 1) surfactant concentration at constant bacterial count; 2) bacterial count at constant surfactant concentration; 3) river water storage period; 4) influence of additional easily assimilated COD; 5) temperature; 6) agitation; and 7) oxygen partial pressure of aerating gas.

BIODEGRADATION OF NONIONICS. C. A. Vath (Union Carbide Corp.). *Soap Chem. Specialties* **40**(2), 56–8, 182 (1964). This is the first part of a discussion of the basic concepts of biodegradation; physical, chemical and biological tests for measuring biodegradation under various environmental conditions; the relative degradabilities of ethoxamers made from several straight-chain hydrophobe sources.

HEAVY DUTY DETERGENT COMPOSITION. R. A. Grifo, R. L. Mayhew, A. Stefcik and F. E. Woodward (General Aniline & Film Corp.). *U.S. 3,122,508.* An alkaline cleaning composition in the form of a 0.3% by weight aqueous solution and having a pH of 3–12 consists of approximately 1 to 10 parts of an alkali metal polyphosphate and 1 to 10 parts of the reaction product of a mixture of primary and secondary phosphate esters of P_2O_5 with a nonionic surface active agent. The nonionic agent has the molecular configuration of a condensation product of at least 1 mole of ethylene oxide and up to an amount sufficient to provide the agent with about 95% by weight of combined ethylene oxide with 1 mole of a compound containing 6–150 carbon atoms and a reactive hydrogen atom. This compound may be selected from the group consisting of phenol, alkyl phenols, aliphatic alcohols, fatty acids, fatty amines, fatty amides, rosin amines, long chain sulfonamides, long chain-substituted aryl sulfonamides, and high molecular weight mercaptans.

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have been involved in analytical work and quality control for many years have had nightmares contemplating the probable future. The specifications for a future fat may be something like this: 12:12:12 (trilaurin), 14:14:14 (trimyristin), 16:16:16 (tripalmitin), and 18:18:18 (tristearin) in equal quantities. You won't be able to hedge because the customer will also have a gas chromatograph. Someone once said gas chromatography will make honest men out of us all.

Fatty acids are the starting material for a great many derivatives. These include long chain alcohols, aldehydes, nitriles, amines, ketones, amides, and quaternary ammonium compounds. Gas chromatography has been applied to the separation and analysis of each of these materials. Before GLC, it was necessary to develop a functional group analytical method for each new compound as they were made. Very often, more time was required to find a suitable analytical method than it took to develop the original compound. It goes without saying, that an enormous amount of time is being saved in research projects through the use of GLC. Another dividend, for the analytical chemist at least, is the ease of convincing the organic chemists and other researchers their compounds are not always pure. When one observes a dozen peaks in a chromatograph of a "pure" compound, its purity becomes suspect.

Up to now we have only discussed direct GLC applications in the lipid field. There are many other analytical applications for GLC of interest to the fat and oil industry. These applications include investigations on pesticides in fats, chick edema factor, flavors, odor and color problems. The great sensitivity of GLC will probably make the solution of these problems possible or at least somewhat easier.

Gas chromatography complements many other analytical methods. Used in combination with other instrumental techniques, the scope of each method is increased. The effluents from the GLC column can be further examined using IR and UV absorption spectrometers. Another very useful technique is to pass part of the gas stream directly into a mass spectrometer. Radioactive fatty acids and esters have been chromatographed and their radioactivity measured as they emerge from the column. The effluents of the gas stream may be passed into chemical reagents to indicate functional groups. Another common practice is to collect the peaks for further characterization by chemical and physical means. Many devices have been invented for this purpose. Some are as simple as a glass capillary in a paper cup of dry ice. Others are complex electronically controlled fraction collectors capable of automatically collecting up to 50 peaks. Once a peak is collected, a melting point may be run on it or perhaps IR or UV spectroscopy analysis. If the collected peak is radioactive, its radioactivity may be measured with a liquid scintillation counter. From all this, one can surmise rightly that it often takes more than a retention time to prove the identity of an unknown gas chromatographic peak.

Another useful technique that complements GLC is thin layer chromatography (TLC). Spots from thin layer chromatographs can be recovered and gas chromatographs may be collected and rechromatographed on thin layer plates for proof of purity and identity. It should be mentioned that the older techniques of column and paper chromatography can also be used in much the same way as TLC to complement GLC. However, these earlier methods cannot match the resolving power or ease of operation of either GLC or TLC.

Since the earliest days of gas chromatography, it has always been possible to collect enough material from an analytical chromatograph for further analytical work. Investigators soon visualized scaling up GLC to obtain significant quantities of very pure substances. At first these early preparative instruments were nothing more than scaled up plumbing. Columns of one to ten inches in diameter were often constructed. It was quickly found that though these large columns had great capacity, their efficiency was usually very poor. The modern approach is to use smaller diameter columns with automatic injection and collection. Such an instrument may be programmed to run continuously until considerable quantities of the desired pure substances are collected. A number of such instruments are available commercially. Unfortunately other factors can complicate preparative GLC. These are column bleed, enrichment of trace impurities, and contamination with late appearing peaks in the automated instruments. It may be worthwhile commenting on some of these effects. When we first began to use automated preparative gas chromatography on fatty materials, we collected the desired peak over and over from many sample injections until a considerable quantity was obtained. Analytical gas chromatography was then used to assay the material. It was with considerable dismay that we found we had not the single *pure* compound as we expected. We had enriched some trace materials that emerged very close to the main peak and had an obvious mixture. Compounds that were normally only in trace amounts, were now present in appreciable quantities. This trace enrichment could be used to advantage to obtain the compounds, but it can complicate the purification process.

The above mentioned problem has another variation in that materials with much longer retention time can contaminate the peaks being collected. This happens with instruments that program through a preset time cycle. If

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