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The Determination of Polymers in Fats and Oils

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Abstract

Fats and fatty acids are polymerized by oxidative or thermal processes. Structures have been deduced by using a number of chemical and physical techniques. General methods applicable to the analysis of polymerized oils include determinations of acetone number, iodine value (I.V.), molecular weight, dielectric constant, viscosity, and refractive index. Monomers, dimers, and trimers are separated generally by molecular distillation. In addition, urea fractionation and a number of chromatographic techniques are useful for the detection of monomers, dimers, and polymers.

Introduction

THE ANALYSIS of polymers present in fats and oils is of practical importance to both the chemist and the nutritionist. Knowledge of the chemistry and structure of polymeric products has led to the introduction of new industrial raw materials. On the other hand, nutritionists are interested in the nutritive value of substances formed in heated and polymerized fats.

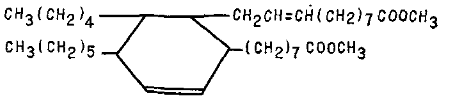
The purpose of this paper is 1), to survey the various structures found in heated and polymerized fats, noting procedures used for isolation and analysis; and 2), to describe techniques and methods useful for determining polymers in fats and oils.

Polymers are formed in fats and oils by processes which may be described as either thermal polymerization or oxidative polymerization. Thermal polymerizations proceed in the absence of oxygen. They involve primarily Diels-Alder addition of the double bond system at the 1, 4- position of a conjugated diene structure to form hydroaromatic cyclic compounds. On the other hand, oxidative polymerizations occur by free-radical mechanisms. Hydroperoxides, formed initially at low temperatures, decompose on heating to form principally dimeric products whose monomeric units are linked through carbon. Newman (1) and Perkins (2) have reviewed the chemical and nutritional changes that occur in heated fats. Sonntag (3) has reviewed reactions occurring during thermal polymerization of fatty acids. Privett (4) discussed autoxidation and oxidative polymerization of drying oils.

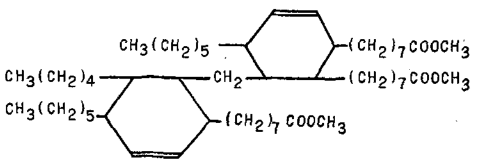
Experimental

Thermal Polymerization

Dimers and polymers. Bradley and coworkers (5, 6,7) observed changes in viscosity, molecular weight, iodine number, refractive index, density, and saponification number of methly or ethyl esters derived from thermally polymerized oils. On the basis of physical and chemical data obtained, the authors concluded that Diels-Alder addition reactions produce monocyclic and bicyclic dimers from linoleate and linolenate respectively, and further Diels-Alder addition produces trimeric cyclic structures. Paschke and coworkers (8,9) found that linoleate dimerizes largely by thermal conjugation followed by Diels-Alder addition of conjugated isomer with nonconjugated linoleate. Dimers and trimers produced from heat polymerization of methyl linoleate are represented by the following structures:



DIMER

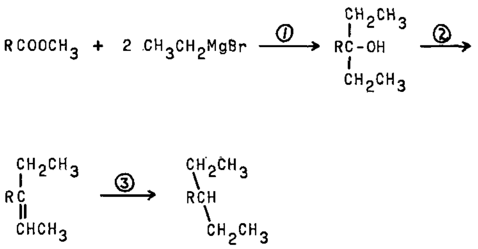


TRIMER

Chin (10), and Clingman and coworkers (11) presented formal proofs of the presence of six membered rings in the dimers isolated from thermally polymerized methyl eleostearate. Chin dehydrogenated the dimers with selenium and obtained derivatives of benzene and naphthalene. Clingman and coworkers, by substitutive bromination-dehydrobromination and oxidation obtained a 9% overall molar yield of methyl prehnitate (1,2,3,4-benzene-tetracarboxylate) from eleostearate dimers, indicating that the eleostearate dimer was a tetrasubstituted cyclohexene derivative.

The methyl eleostearate dimer that Chin isolated from polymerized tung oil had a molecular weight (Rast) of 577. Hexanoic and azelaic acids were recovered from the dimer oxidized with potassium permanganate and ozone, which suggested that these residues were present in the side chains.

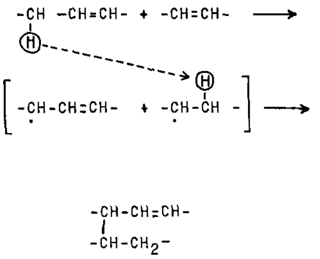
Waterman and coworkers (12,13,14,15) used refractive index measurements to determine the number of cyclic structures (rings) in the acyl groups of polymerized oils. Methyl esters derived from polymerized samples were converted to saturated hydrocarbons by 1) reaction with Grignard reagent followed by hydrolysis; 2) dehydration; 3) hydrogenation; as follows:



$$RCOOCH_3 + 2\,CH_3CH_2MgBr \xrightarrow{①} RC(OH)(CH_2CH_3)_2 \xrightarrow{②} RC(CH_2CH_3){=}CHCH_3 \xrightarrow{③} RCH(CH_2CH_3)CH_2CH_3$$

The saturated hydrocarbons were then analyzed for average number of rings per molecule from measurements of refractive index, specific gravity and average molecular weight. From the molecular weight and calculated specific refraction, the average number of rings per molecular was obtained by reference to published graphs. Waterman and coworkers (15, 16) also determined the average number of rings present in polymerized glycerides and methyl esters by analysis of the saturated hydrocarbons obtained by direct high pressure hydrogenation.

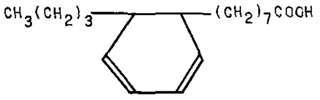
Diels-Alder addition may not be the sole mechanism of thermal polymerization. Sutherland (17) proposed a mechanism whereby a double bond of one monomer unit abstracts a hydrogen atom from a methylene group of another monomer unit, and the two molecules then unite to create a dimer linked through a carbon to carbon bond:



Norton and coworkers (18) isolated dimers from thermally polymerized linoleate (1 hr, 300C, in vacuo) that contained a considerable amount of conjugated unsaturation. Yields of prehnitic acid (3%) were no greater than from dimers isolated from long heated (12 hr) linoleate. The authors stated that these results were in accordance with the hypothesis that Diels-Alder type addition is not the sole dimerization mechanism. Paschke and Wheeler (8) obtained a dimer from oleic acid heated at 300C, containing one double bond.

Cyclic monomers. Monomers presumed to be cyclic were isolated from thermally polymerized methyl linoleate (8,9). The monomers did not hydrogenate to methyl stearate. Mehta and Sharma (19) obtained a monomeric methyl ester from thermally polymerized safflower oil which did not adduct with urea. It was presumed that this material was a cyclic product of methyl linoleate.

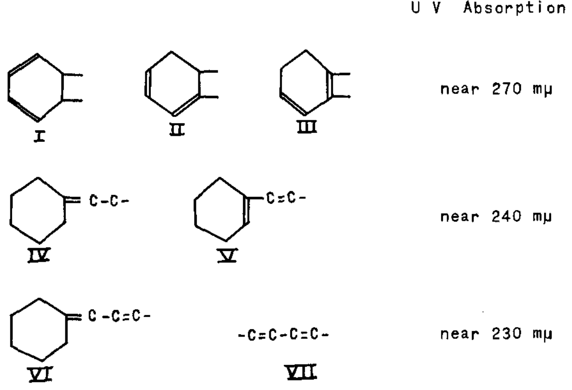
Rossmann (20) demonstrated earlier that cyclic monomer was produced from thermal polymerization of β-eleostearic acid. He proposed the following structure:



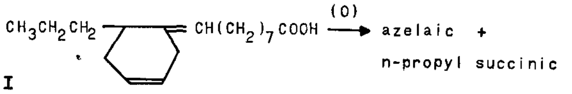
The monomeric material isolated by vacuum distillation and low temperature crystallization was aromatized by heating with nitric acid, and then oxidized with permanganate. The preparation of other aromatic derivatives, elementary analysis, and determination of molecular refraction and iodine number established the cyclic nature of this material.

Paschke and Wheeler (21) prepared cyclic monomers from methyl eleostearate by heating a 10% solution in methyl laurate at 250C for 48 hr in a sealed, evacuated ampoule. Methyl laurate was distilled off through a short column at reduced pressure, and the cyclic monomers were isolated from the residue by urea adduction followed by distillation to separate them from the dimers and polymers. The cyclic monomers exhibited a peak at 241 mμ in the ultraviolet and peaks at 13.3 and 14.3μ in the infrared. Chemical aromatization followed by oxidation resulted in a 20% yield of phthalic anhydride. The authors concluded that the cyclic product consisted largely of an ortho disubstituted cyclohexadiene.

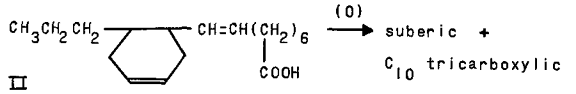
Rivett (22) also isolated cyclic monomers from polymerized methyl eleostearate. Monomeric material, separated in a falling-film molecular still, was adducted with urea. The nonadducting monomers were converted to orthophthalic acid (17% yield) by substitutive bromination-dehydrobromination, and oxidation with potassium permanganate. The cyclic monomers were also examined by infrared and ultraviolet spectrophotometry. A strong band at 10.13μ in the infrared spectrum was due to conjugated *trans-trans* diene. A strong band at 14.3μ was said to be due to substituted cyclohexadienes. Ultraviolet spectra showed peaks or shouders at 232 mμ, 240 mμ, and 268 mμ. The following possible conjugated diene structures were postulated:



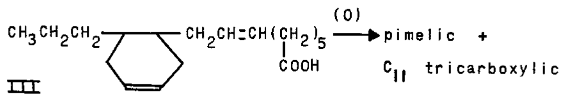
MacDonald (23) isolated monomers with cyclic structures from thermally polymerized linseed oil. He suggested that infrared absorption at 15.2μ was due to the presence of a cyclohexene ring. Subsequently, McInnes and coworkers (24) presented further evidence for the presence of cyclic monomers in thermally polymerized linseed oil. The distillable esters which did not form adducts with urea were separated into three fractions by gas chromatography at 240C on an 8 ft column of Apiezon M-Celite 545 (1/4,w/w). Examination of infrared spectra showed that the band at 15.2μ, attributed to C-H out-of-plane deformation of a *cis*-disubstituted double bond in a 6-membered ring, was absent in fraction 1, but present in fractions 2 and 3. The products obtained by oxidation of the esters with periodate-permanganate were analyzed by gas chromatography and paper chromatography. The results suggested a number of structures, as follows (only the dicarboxylic acids were identified definitely):



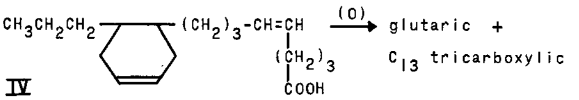
I



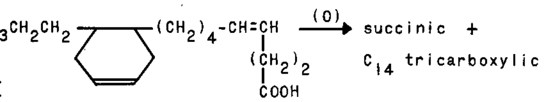
II



III



IV

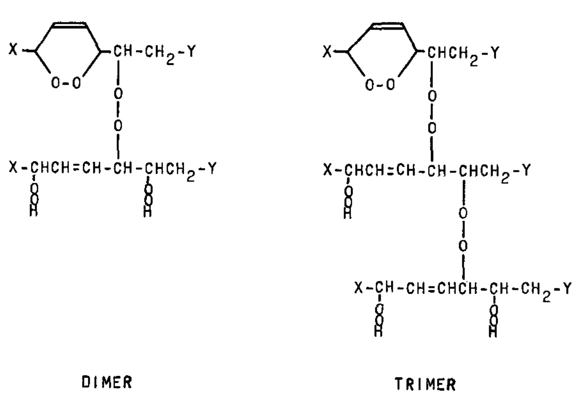


V

Analysis of the oxidation products indicated that structure I was the major component of fraction 1. The authors suggested that the presence of a large percentage of this structure in fraction 1 would account for the lack of absorption at 15.2μ. The presence of a double bond attached directly to the ring of structure I might hinder C-H out-of-plane deformation of the ring double bond and prevent absorption at 15.2μ. It was also suggested that low yields of phthalic anhydride obtained after substitutive bromination-dehydrobromination and oxidation could be accounted for by the spacial configurations of the proposed structures. The steric requirement for dehydrohalogenation in a 6-membered ring, that the halogen and hydrogen atoms must be trans-diaxially oriented in the transition state (25), could not easily be fulfilled by these structures.

Oxidative Polymerization

Air oxidation of polyunsaturated fatty acids at temperatures below 100C (autoxidation) results in the formation of conjugated hydroperoxides. Decomposition of hydroperoxides at low temperatures produces free radicals which can in turn react to form polymers, the monomer units of which are linked through carbon to oxygen bonds. Holman (26) has presented a comprehensive review of autoxidation of fats. Chang and Kummerow (27) autoxidized ethyl linoleate at 30C and isolated polymeric fractions by solvent extraction. The polymers were readily depolymerized with halogen acids at room temperature to monomers containing carboxyl and hydroxyl groups. Dimer and trimer resulting from low temperature autoxidation of linoleate may be represented as follows (2):



When fatty acid hydroperoxides are heated to about 100C or above, dimers and polymers are formed that contain carbon to carbon linkages between the monomeric units.

Williamson (28) autoxidized methyl linoleate at 33C while exposed to ultraviolet light, and then decomposed the oxidized material by heating in a nitrogen atmosphere at 100C. The dimers, isolated by solvent fractionation and molecular distillation, contained monomeric units linked by carbon to carbon bonds. The trimers were reduced with hydrogen iodide at room temperature to monomer and dimer, suggesting that this material consisted of three monomeric units, two of which were linked by carbon to carbon bonds, and the third by oxygen linkages.

Frankel and coworkers (29) decomposed autoxidized triglycerides and methyl esters, and purified hydroperoxides by heating at 210C in a nitrogen atmosphere. Monomers, dimers, and polymer were isolated by molecular distillation of the methyl esters. The dimers, containing about two double bonds per mole [Wijs and hydrogen iodine values (I.V.)], were not split with hydrogen iodide or by catalytic hydrogenation. It was suggested that intramolecular peroxide groups might be present. Oxidation with periodate-permanganate yielded a complex mixture of mono- and dibasic acids. The dimers could not be aromatized by substitutive bromination-dehydrobromination.

Perkins and Kummerow (30) prepared thermally polymerized corn oil by bubbling air through the oil at 200C. Fatty acids from the heated oil were fractionated by urea adduction. The nonurea adduct-forming fraction was distilled in a falling-film molecular still, and nondistillable material was fractionated by solvent extraction. The insoluble acids, after conversion to methyl esters, were subjected to additional solvent extraction, while the soluble acids were redistilled. Dimer and polymer fractions were isolated with a molecular weight range of 692 to 1600. The dimers and polymers had a high content of carbonyl and hydroxyl groups and could not be aromatized, indicating the absence of cyclic structures.

Firestone and coworkers (31) heated cottonseed oil at 205 and 225C in the presence of air. The dimers and higher polymers contained only moderate amounts of carbonyl and hydroxyl groups. Several percent of nonurea adduct-forming monomers were also isolated. These monomers could not be aromatized; however, the dimers, after bromination-dehydrobromination and oxidation, absorbed in the ultraviolet region at 250–260 and 270–280 mμ, suggesting the presence of cyclic structures. Paschke and Wheeler (21) had previously suggested that oxidized polyunsaturated fatty acids might undergo cyclization.

Discussion

Methods for Determining Polymers in Fats and Oils

General Chemical and Physical Methods. Determinations including iodine number, saponification value, molecular weight, dielectric constant, viscosity, refractive index, specific gravity, etc. do not determine the polymer content of samples directly. They are useful, however, for process control, for following the course of polymerizations, and for estimating the extent of polymerization of individual samples. Chemical and physical tests used generally for analyses of fats and oils are discussed by Mehlenbacher (32). General methods of analysis for drying oils were reviewed by Link (33). Several physical methods useful for examining thermally and oxidatively polymerized oils have been described by O'Hare and coworkers (34). Täufel and coworkers (35) have reviewed the application of a number of chemical and physical methods to analysis of polymerized fats.

The decrease in unsaturation (determined by catalytic hydrogenation) has been used to estimate the extent of thermal polymerization of vegetable oils (36). Viscosity is frequently used as an index of polymerization. Sims (37) studied the relationship between viscosity and content of polymeric glycerides and polymeric acyl groups in thermally polymerized oils. The oils studied all differed in their viscosity—polymeric glyceride relationships. Polymer contents were determined by molecular distillation.

Flory (38) showed that for linear polymers, an approximately linear relationship exists between the square root of the weight-average molecular weight and the logarithm of the absolute viscosity. Bernstein (39) found that a linear relationship was obtained when fractions of thermally polymerized oils, extracted with a homologous series of normal alcohols, were examined. Molecular weights (number-average) was determined cryoscopically. Paschke and Wheeler (40) observed that the logarithm of viscosity of thermally polymerized linseed oils was proportional to the square root of the number-average molecular weight. Since viscosity is more nearly a function of weight-average molecular weight, the oils studied had a fairly constant ratio of weight-average to number-average molecular weight in the polymeric glycerides.

Plots of log viscosity versus square root of molecular weight for several oxidatively polymerized cottonseed oils prepared by the author are shown in Figure 1. Linear relationships were obtained. The oil heated in the presence of air had a greater slope than the two air-blown oils.

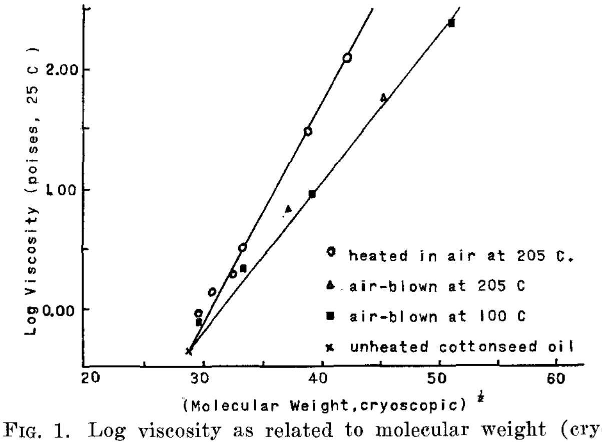


FIG. 1. Log viscosity as related to molecular weight (cryoscopic, in cyclohexane) of oxidatively polymerized cottonseed oils.

Molecular weights are determined generally by cryoscopic, ebullioscopic, or vapor pressure methods, which yield number-average molecular weights (sample weight divided by the number of molecules in the sample. Methods yielding weight-average molecular weights have also been used to examine polymerized oils. Walker and coworkers (41) determined the weight-average molecular weight of thermally polymerized linseed oil by light scattering. The whole oil had a molecular weight of 32,000 whereas an acetone-insoluble fraction had a molecular weight of 87,000. Lück and coworkers (42) determined the weight-average molecular weights of several polymerized linseed oils by ultracentrifugation, using a capillary-type synthetic boundry cell. The molecular weight of a thermally polymerized oil (111 poise) in hexane solvent was 11,900 compared to 7500 in ether, suggesting that either association of the molecules occurs in hexane, or that association occurs in both solvents, but to a greater extent in the nonpolar solvent. Donnelly (43) investigated the use of equilibrium ultracentrifugation for the study of molecular weight distribution in methyl esters derived from oxidatively polymerized cottonseed oil.

Solvent Fractionation. Solubility differences in various solvents have been applied by many workers to the fractionation of polymerized oils. McQuillen and Woodward (44) used hot and cold acetone to fractionate thermally polymerized oils. Privett and coworkers (45) observed that acetone could be used to separate polymerized oils into a number of fractions with widely differing properties. Mehta and Sharma (46) examined a number of thermally polymerized linseed oils by extraction with 5 volumes of cold acetone. Soluble and insoluble portions were separated. The acetone soluble portions were essentially monomeric triglycerides with molecular weights varying from 918 to 950. Walker and coworkers (41) compared the fractionation of thermally polymerized oils by molecular distillation and room temperature solvent extraction with acetone. Similar results for monomer and polymer glycerides were obtained by both procedures.

Bernstein (39,47) recommended using a homologous series of normal alcohols to separate thermally polymerized oils into a number of fractions of increasing molecular weight. Fractionation of polymerized linseed and soybean oils were performed to determine the distribution of monomeric, dimeric, and polymeric glycerides in the oils. Polymeric glycerides as high as the heptamer were extracted from polymerized linseed oil. Sims (36) fractionated several polymerized linseed and tung oils using Bernstein's method in order to determine the approximate polymer distribution in these oils.

Kaufmann and coworkers (48) published a paper chromatographic procedure for examining polymerized oils in which samples were fractionated by developing first with 99% methanol to separate free fatty acids, and then with 99% acetone to separate unpolymerized glycerides and lower polymers. Cobalt acetate and Rhodamine B were used to detect the spots. Walker and coworkers (41) used additional solvents (water-saturated n-butyl alcohol, n-hexyl alcohol, and n-hexane) to obtain a number of zones with successively higher glyceride polymers.

A paper chromatogram of several heated cottonseed oils prepared by the author is illustrated in Figure 2. This technique may be useful for examining highly polymerized oils, but is not sensitive or reproducible

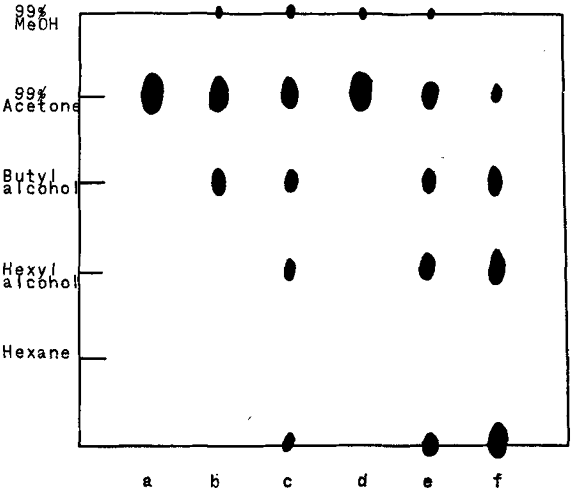


FIG. 2. Paper chromatogram of oxidatively polymerized cottonseed oil. a) fresh oil, b) air-blown 56 hr at 205C, c) air-blown 72 hr at 205 C, d) heated in presence of air 96 hr at 205C, e) heated in presence of air 307 hr at 205C, f) heated in absence of air 125 hr at 300C.

enough for quantitative determinations of polymer content.

Two procedures for detecting thermally polymerized fats, based on the insolubility of polymerized triglycerides in various solvents, are described in the German Standard Methods of Analysis for Fats, Fat Products, and Allied Materials (49). In the first procedure, the insolubility of polymerized fats in n-propyl alcohol is observed. Absolute n-propyl alcohol is added to 2 ml of sample to give a total volume of 20 ml. The mixture is shaken, and a turbid solution or lower insoluble layer of oil results when any appreciable amount of polymer is present. In the second procedure, sample and standards are chromatographed on paper with a 1/1 mixture of acetone-methanol. Polymerized oils remain at the origin.

The AOCS acetone tolerance test (50) is used in this country to estimate the high polymer content of thermally polymerized drying oils. Acetone is soluble in unpolymerized, but not in polymerized oils. This test determines the weight of acetone in grams which will dissolve in 30 g of oil before a permanent cloudiness is produced. The acetone number is defined as the weight of anhydrous acetone in g required to produce a cloudiness in 100 g of oil at 25C.

Molecular distillation. Molecular distillation takes place from a quiet surface and there is no agitation due to boiling. Molecules evaporate from the surface of a liquid, and are trapped at a cooled condenser. There should be a minimum of obstruction in the path between distilland and condenser, and the distance between distilland and condenser should be less than the mean free path of the vaporized molecules. Rate of evaporation is controlled by the rate at which molecules can escape from the distilland.

Factors controlling the rate of evaporation include pressure of surrounding atmosphere, temperature, and molecular weight of components of the sample. Langmuir (51) derived an equation for the rate of evaporation of a substance, as follows:

$$n = pA\sqrt{1/2\pi MRT}$$

where n is the number of moles evaporating from a surface of A cm^2, p is the vapor-pressure in dynes/cm^2 at the evaporating surface, T is the absolute temperature, M is the molecular weight and R is the gas constant (8.314 joules/deg./mole). Burrows (52) has described in detail the theory, design, and operation of molecular distillation equipment. Weissberger (53) has presented a comprehensive survey of distillation theory and technique.

Although it is a poor means of fractionating materials, molecular distillation is valuable for isolating whole classes of substances, and is the most satisfactory means of isolating monomers, dimers, and polymers from lipids.

Molecular distillation can be carried out at lower temperatures than with other distillation methods, and therefore with the least risk of thermal decomposition. This risk is minimized with the use of centrifugal molecular stills in which the sample is in contact with a hot rotor for only a fraction of a second.

According to Carney (54) there are four general types of molecular stills: 1) pot still, 2) tray still, 3) falling film still, and 4) centrifugal still. In a pot or tray-type molecular still, distillation takes place directly from a heated pool or layer of distilland. Falling film and centrifugal stills handle larger volumes of sample than pot stills. Falling film stills contain two concentric tubes; one tube serves as an evaporative surface while the other serves as the condenser. The distilland is added to the still so that it flows over the heated evaporative surface, forming a thin film. Distillate is collected at the condensing surface and flows into a receiver while undistilled material is col-

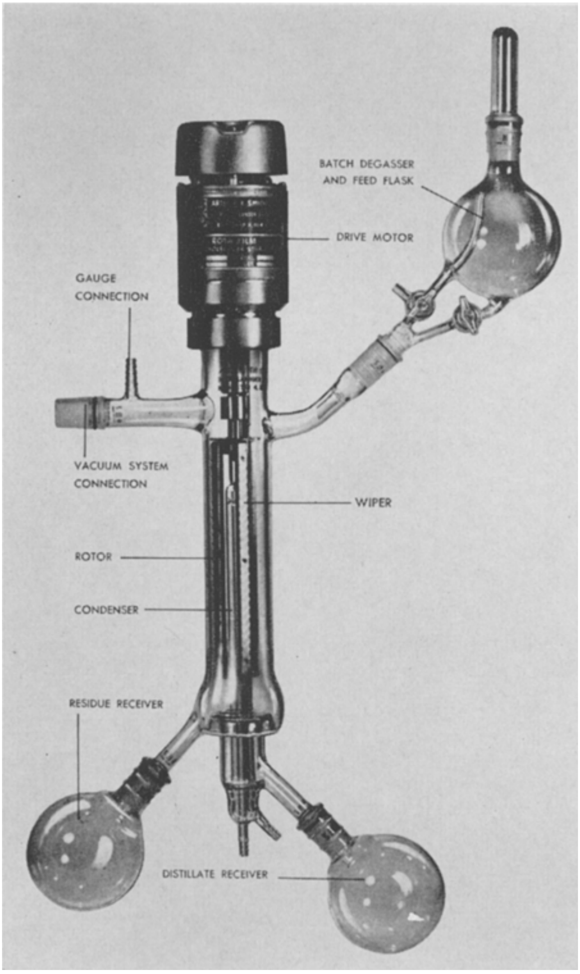


FIG. 3. Falling film molecular still (A. F. Smith Co., Rochester, N. Y.)

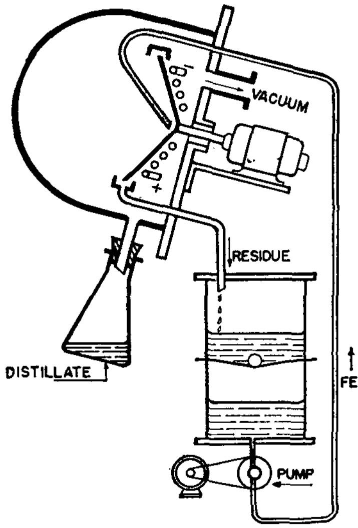


FIG. 4. Centrifugal molecular still (55). Courtesy of Analytical Chemistry.

lected in another receiver. A falling film still with rotating wiper blades is shown in Figure 3. The blades spread the sample into a thin film as it accelerates the flow of sample down the heated evaporative surface. Total contact time at temperature of evaporation is less than one second. The wipers also keep the evaporative surface free of deposits.

The advantages of a thin film and very short heating time are important features of the centrifugal molecular still, (55) shown in Figure 4. A heated centrifugal cone is used to spread the distilland into a very thin turbulent film, which travels across the heated surface in a fraction of a second. Residue is flung off the cone into a collecting gutter while the distillate condenses on the inner surface of the surrounding bell jar.

In addition, fractionating molecular stills have been devised to give better separations than can be obtained with pot or falling film stills. An example is the high vacuum brush-type still (Consolidated Electrodynamics Corp., Rochester, N. Y.) in which the distillate from a heated pot condenses on an air-cooled stainless steel brush inside a heated glass column and is flung out to the heated surface of the column. Fractionation is accomplished by reevaporation and recondensation over the length of the column, the lightest fraction of distilland rising to the top where it is condensed and collected.

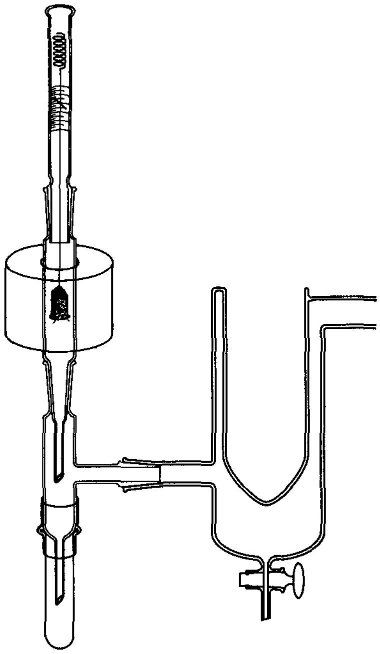


FIG. 5. Micromolecular still (59).

A number of micromolecular stills have been devised for rapid determination of monomers, dimers, etc. in polymerized systems. Rushman and Simpson (56) designed a micro still for quantitative use in which a small hotplate was incorporated into the unit. A small dish containing the sample was weighed before and after heating for a specified time at suitable temperatures.

Booy and Waterman (57) devised a micro still consisting of a shallow pan suspended from a glass helix enclosed in an evacuated glass tube. Heat was supplied by an infrared lamp positioned at a short distance from the still. An accuracy of 1% was obtained with known mixtures of methyl esters, using the glass helix for continuous weighing during the distillation. Sims (58) constructed a micromolecular still for analytical distillation consisting of a glass pan suspended from a quartz helix. The pan was surrounded with an internal heating coil, and an internal thermocouple was used to estimate sample temperature. The course of distillation was followed by measuring the change in helix extension using a cathetometer.

Paschke and coworkers (59) described an apparatus in which heat was supplied by a thermostatically controlled aluminum block surrounding the distillation area. This apparatus (Figure 5) consisted of a small bale of glass wool to disperse the sample, suspended from a quartz helix. The glass wool prevented splattering and gave a large distillation surface. Up to 0.5 g of methyl esters or more were analyzed for monomer, dimer, and polymer using a bale of suitable size. A cathetometer was used to measure changes in helix extension. Pressures of 1–5μ were used for the distillation. Monomer was distilled at a block temperature of 150C in about 30 min. Dimers were distilled at a block temperature of 250C in about the same time. Known mixtures of monomer, dimer, and trimer methyl esters were used to determine the proper distillation temperatures. Good agreement was obtained when results were compared with those obtained by the alembic pot method of Cowan and coworkers (60).

Results of analyses in the author's laboratory of four methyl ester samples with this micro still, including a commercially available (Emery Industries, Cincinnati, Ohio) thermally polymerized dimer and trimer of linoleic acid, are shown in Table I. The samples were analyzed at temperatures of 125 and 225C, and pressures of 1–2μ (McLeod guage).

Cold-finger pot stills or sublimators are useful for determining total polymers in methyl ester samples. A sublimator with a well for solid CO_2-acetone is used routinely in the author's laboratory. The distance from distilland surface to cold finger condensing

TABLE I
Micromolecular Distillation of Methyl Esters

%	Cottonseed oil	Methyl 12-hydroxystearate	Thermal dimer	Thermal trimer
Monomer	99.5	99.5	3.2	0.0
Dimer	0.5	0.5	96.6	6.3
Trimer	0.0	0.0	0.2	93.7

TABLE II

Vacuum Sublimation of Methyl Ester Samples

%	Methyl stearate	Methyl 12-hydroxy-stearate	Methyl stearate-methyl 12-hydroxy-stearate (1:1)	Thermal dimer	Oxidative dimer
Distillate..........	99.0	83.8	97.5	2.3	1.2
Residue...........	1.0	16.2	2.5	97.7	98.1

surface is ¼ in. A bank of 6 sublimators connected to a manifold permits multiple sublimations. Table II shows results of vacuum sublimation of individual 100 mg samples of several methyl esters, at 85C and 50μ pressure for 1.5 hr. Although only 83.8% of methyl 12-hydroxystearate was sublimed under these conditions, a 1/1 mixture of the hydroxy ester with methyl stearate was recovered in the distillate in 97.5% yield. Sublimation is a convenient means of determining total dimers and polymers in methyl ester samples while simultaneously eliminating these materials from the monomeric esters prior to gas chromatography.

Urea Adduction. The reaction products from thermally polymerized oils (46,61,62) and oxidatively polymerized oils (30,31) have been separated from the unchanged fatty acids by urea adduction. Recently, a procedure has been developed for determining nonurea adducting acids (urea filtrates) in fats and fatty acids (63). Powdered urea is added at room temperature to unsaponifiable-free methyl esters dissolved in a minimum of methanol. The resultant slurry or powder is allowed to stand for 2 hr and is then filtered and washed with fixed volumes of urea-saturated methanol. A correction is made for small amounts of adduct decomposed during filtration by subtracting the weight of urea filtrate in a second wash from that obtained in the first wash.

Generally, urea adduction provides an estimate of dimers and trimers, etc. in thermally oxidized or polymerized oils since the dimers and polymers constitute the bulk of the nonadducting esters present. However, cyclic or other nonadducting monomers may be present in oils, and these materials are also isolated with the dimers and polymers. Nevertheless, urea adduction is useful for estimating total alteration of unsaturated fatty acids in oxidized and polymerized oils.

The total urea filtrate (as methyl esters) formed in cottonseed oil heated in the presence of air at 205C (31) is plotted in Figure 6 as a function of time of heating. The total quantity of urea filtrate monomers, dimers, and trimers at any heating interval approximately equals the total urea filtrate esters found.

TABLE III

Analysis of Methyl Esters of Heated Cottonseed Oil (192 hr, 225C)

	Micromolecular distillation		Sublimation	Urea adduction
	Mol wt.	%	%	%
Monomers......................	286	64.3	65.3	64
Dimers..........................	595	24.8	34.7	36
Trimers.........................	1143	10.9		

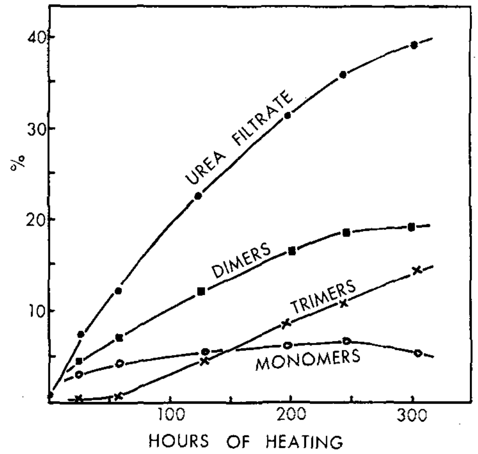


FIG. 6. Urea filtrate from heated cottonseed methyl esters. (31).

A comparison by the author of results obtained by micromolecular distillation, sublimation, and urea adduction of methyl esters derived from cottonseed oil heated 192 hr at 225C in the presence of air is shown in Table III. Filtrate esters from urea adduction are tabulated as total dimers and trimers. Actually, the filtrate esters contain 2–3% of nonadducting monomers. Nevertheless, there is good agreement between the three methods for monomer and polymer content. The molecular weights of the fractions obtained by micromolecular distillation were determined by the isothermal distillation method using a Vapor Pressure Osmometer, Model 301, Michrolab Inc., Mountain View, Cal.

Column Chromatography. A liquid-partition chromatographic method was developed by Frankel and coworkers (64) to determine dimers in fats. Silicic acid treated with 20% methanol served as the immobile phase. Fatty acid samples (0.1–0.2 g) were eluted with 2% methanol in benzene to yield first, a monomeric fraction, and then, a dimeric fraction. A polar fraction was then eluted with ethyl ether. Chromatographic separation of the acids was followed by titration with 0.2*N* alcoholic KOH to a thymol blue end point, using a microburette.

The method was used by Frankel and coworkers to determine the oxidative dimer content of deodorized soybean oils. Thermally polymerized acids were found to elute in the same position as oxidative dimer acids. Chromatographic separation of thermal dimer and polymer is shown in Figure 7. The authors stated that the method has a greater sensitivity than distillation and will determine small amounts of either monomer or dimer in the presence of larger amounts of one over the other.

Paper Chromatography. A paper chromatographic method for detecting small amounts of thermal dimer and trimer acids in fats and oils was developed recently by Rost (65). The procedure involves: a) saponification of glycerides and isolation of total fatty

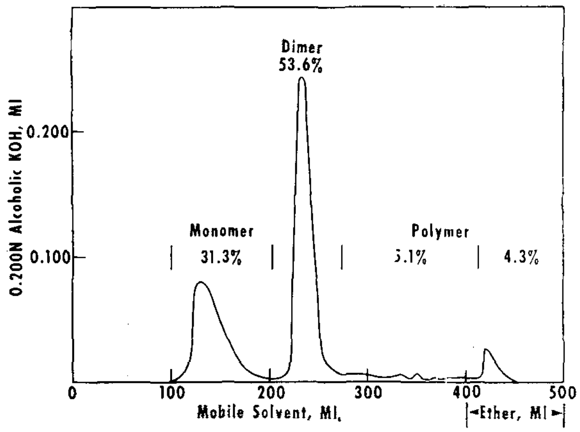


FIG. 7. Column partition chromatographic separation of dimer and polymer from thermally polymerized conjugated linoleic acid (64).

TABLE IV
Paper Chromatography of Fatty Acids, Thermal Dimer and Trimer of Linoleic Acid (65)

Component	R_f	Component	R_f
Lauric acid	0.56	Oleic acid	0.29
Myristic acid	0.43	Stearic acid	0.15
Linoleic acid	0.42	Linoleic dimer acid	0.12
Palmitic acid	0.30	Linoleic trimer acid	0.00

acids; b) enrichment of dimer and trimer acids; c) paper chromatography; and d) quantitative determination of the dimer acids.

Enrichment of dimer and trimer acids was accomplished by urea adduction. Saturated fatty acids were almost completely removed by adducting 1 part of sample with 4 parts each of urea and methanol. When fats and oils containing large amounts (over 40%) of high melting fatty acids were examined (*e.g.*, palm and rapeseed oil), the major portion of saturated acids were removed by low temperature crystallization in methanol prior to urea adduction. Higher unsaturated fatty acids present in fish oils interfered with the paper chromatographic detection of dimer acids. These components were eliminated by hydrogenation prior to urea adduction and paper chromatography.

Enriched dimer and trimer acids were chromatographed by the method of Kaufmann and Nitsch (66) using a petroleum fraction (b.p. 190–220C) as the immobile phase, and 90% acetic acid as the mobile phase. R_f values of the dimer and trimer acids together with R_f values of several normal acids are shown in Table IV.

Spots were developed with copper acetate and sodium diethyldithiocarbamate. Dimeric acids were determined quantitatively by cutting out the colored spots, extracting with ethyl alcohol, and measuring absorption at 435 mμ. The author stated that polymeric components of an oil or fat can be determined down to 0.01% of the total fatty acid content with a precision of 95 ± 5% of the dimer acid actually present. The limit of detection was estimated to be about 20 μg of dimer acid.

Thin-layer Chromatography. This technique has been applied to the analysis of a wide variety of lipid materials. Procedures and applications have recently been reviewed by Mangold (67).

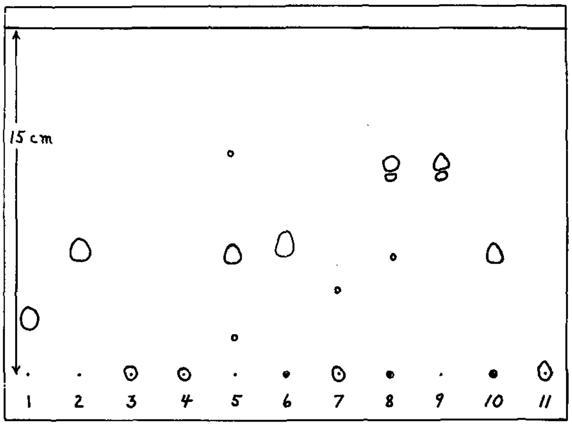


FIG. 8. Thin-layer adsorption chromatography of monomer, dimer, and polymer methyl esters on silica gel (68). Solvent: isooctane-ethyl acetate, 9/1, v/v. Indicator: 10% phosphomolybdic acid in alcohol, followed by heating 30 min at 120C. Amounts: about 100γ, each. 1) methyl 12-hydroxystearate, 2) acetylated methyl 12-hydroxystearate, 3) methyl 9,10-dihydroxystearate, 4) methyl 8,9,15-trihydroxypalmitate, 5) methyl 12-ketostearate, 6) thermal dimer of methyl linoleate (Emery Industries, Cincinnati, Ohio), 7) thermal trimer of methyl linoleate (Emery Industries, Cincinnati, Ohio, 8) methyl esters of cottonseed oil heated 192 hr in the presence of air at 225C, 9) monomer methyl esters from sample (8), 10) dimer methyl esters from sample (8), 11) trimer methyl esters from sample (8).

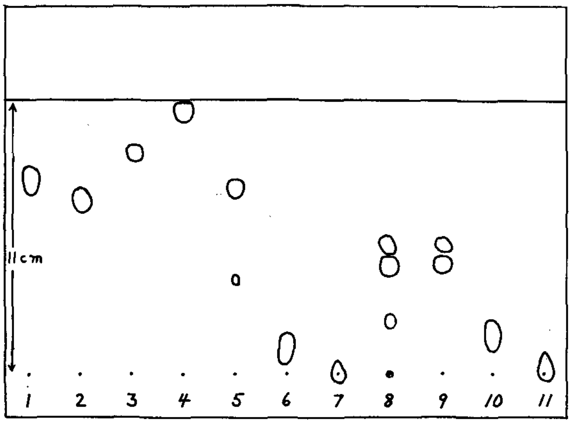


FIG. 9. Thin-layer reversed-phase chromatography of monomer, dimer, and polymer methyl esters on silica gel (68). Solvent: acetonitrile—methyl alcohol—ethyl acetate—water, 2/2/1/1, v/v/v/v. Indicator: 10% phosphomolybdic acid in alcohol, followed by heating 30 min at 120C. Amounts: about 100γ, each. See Fig. 9 for list of samples.

The application of thin-layer chromatography to the separation of monomer, dimer, and polymer methyl esters has recently been investigated in the author's laboratory (68). Samples were resolved by both adsorption and reversed-phase partition chromatography. For adsorption chromatography, Silica Gel G (Research Specialties Corporation, Richmond, California) (1/1 slurry with water) was spread with a 250μ fixed thickness applicator (Brinkmann Instruments, Inc., Great Neck, New York) on 20 × 20 cm glass plates, and dried 1 hr at 105C. The plates were cooled, and spotted with 100 μg of sample, using a 10 μl syringe pipet (Model 701N, Hamilton Company, Whittier, California). The spots were developed for 1 hr with isoocatane-ethyl acetate (9/1, v/v), and dried 45 min in a forced-air oven at 130 C. The spots were identified by spraying with 10% phosphomolybdic acid in alcohol, followed by heating for 30 min at 120C.

Fractionation of a number of oxygenated methyl esters, methyl esters of oxidative and thermal dimers and trimers, and methyl esters derived from heated cottonseed oil is shown in Figure 8. The thermal dimer and trimer were Emery's commercial products, prepared by thermal polymerization of linoleic acid. The cottonseed methyl esters were prepared from cottonseed oil heated in the presence of air for 192 hr at 225C. The oxidative dimer and trimer were isolated by micromolecular distillation of the cottonseed methyl esters. Acetylation of methyl 12-hydroxystearate decreased its polarity and consequently increased its rate of migration. Acetylation of the oxidative and thermal dimers and trimers did not affect their chromatographic behavior.

For partition chromatography, the plates, prepared as described above, were cooled to 5C, and dipped in a cold (5C) 5% solution of DC200 (Dow Corning Corporation, Midland, Michigan) in ethyl ether. Air dried plates, oven-dried at 105C for 1 hr, were spotted as described above, and developed for 1 hr with acetronitrile-methyl alcohol-ethyl acetate-water (2/2/1/1, v/v/v/v). The spots were identified with the phosphomolybdic acid reagent. A chromatogram of the mixture described above is shown in Figure 9. The oxygenated monomers migrated well ahead of and

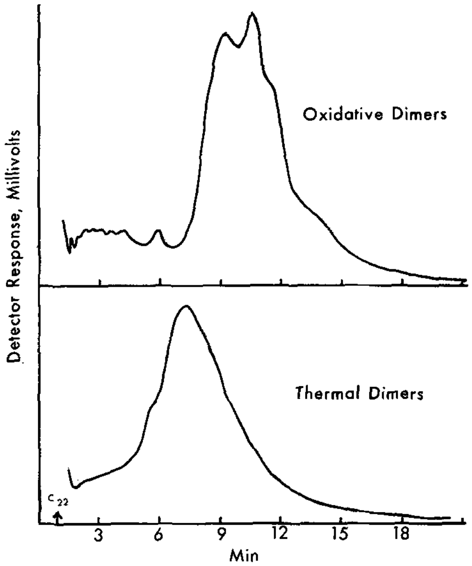


FIG. 10. Gas chromatograms of oxidative and thermal dimers.

were separated from the other components (normal monomeric esters, and oxidative and thermal dimers and trimers). The monomer esters from heated cottonseed oil were separated into two spots (Fig. 9, No. 9), representing oleate and palmitate (lower spot), and linoleate (upper spot).

Gas Chromatography. Although special techniques are required for analysis of high molecular weight compounds, advances in methodology have made it possible to use gas chromatography for routine analysis of a wide variety of these materials. For example, Vanden Heuvel and coworkers (69) used small amounts of very stable substrate (SE-30 silicone rubber from General Electric Co., Silicone Products Dept. Waterford, N. Y. at temperatures around 220C in a commercial gas chromatography with argon ionization detector to separate mixtures of sterols and steroids. Huebner (70) used high temperature chromatography for analysis of glycerides. He analyzed mixtures of synthetic and natural triglycerides using column packings coated with SE-30 liquid phase, and temperature programming from 250 to 400C. These examples of separation of high molecular weight materials suggest that gas chromatography could also be applied to analysis of methyl esters mixtures of monomers, dimers, and trimers. The use of thin coatings of stable liquid phases, short columns, temperature programming, and sensitive detectors should help accomplish this goal.

Preliminary investigation in the author's laboratory has shown that methyl ester dimers from thermally and oxidatively polymerized fats can be chromatographed. Figure 10 shows chromatograms of thermal and oxidative methyl ester dimers obtained with an ionization detection instrument (Chromolab, manufactured by Glowall Corp., Glenside, Pa.). The thermal dimer was prepared by esterifying a sample of Emery's commerical dimer acid, and the oxidative dimers represent a molecularly distilled methyl ester dimer fraction prepared from cottonseed oil heated at 225C for 192 hr in the presence of air. A 6-ft $\frac{3}{16}$ in. o.d. coiled glass column was packed with 1% SE 52 silicone gum rubber on 80–100 mesh Gas-Chrom P (Applied Science Laboratories, State College, Pa.). The column temperature was 296C detector temperature was 270C and outlet argon flow rate, 70 ml/min. Temperature programming should permit the simultaneous analysis of monomer and dimer acids in polymerized fats.

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