Characterization of Used Frying Oils. Part 1: Isolation and Identification of Compound Classes

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In this study, an analytical scheme is presented for detailed qualitative comparisons between heated and unheated oils. This scheme is less subject to loss or alteration of sample components when compared with methods that characterize the distillable non-urea adducting portion of heated oils. In this work, oils were first converted to their corresponding fatty acid methyl esters (FAMEs) by transesterification. These FAMEs were then separated by degree of polarity by means of adsorption chromatography and solid-phase extraction. High-resolution capillary gas and liquid chromatography were used to profile isolated fractions. Mass spectrometric and infrared analyses of major chromatographic features were used to identify the presence of aldehydes, epoxides, ketones, alcohols, phytosterols and dimer methyl esters down to 5 ppm in the original sample.

KEY WORDS: Fatty acid methyl esters, gas chromatography, heated fat, heated fry oil, high-performance liquid chromatography, infrared spectroscopy, mass spectrometry, olestra, sucrose polyesters (SPE).

A number of methods have been reported for the purpose of separating components that have been altered during the heating of fry oils. Early examples include the use of molecular distillation (1,2), urea complexation (3) and solvent partitioning methods (4). More elaborate isolation schemes were developed later. Most common was the method of urea adduction used by Firestone (5), wherein the fatty acid esters are distilled and adducted with urea. The distillable non-urea adducting (DNUA) portion has been used to study the chemical changes taking place during frying (6–8), for characterizing the various chemical components (9,10) or for feeding studies to assess toxicity of heated-fat components (11–13).

It has been pointed out that such separation techniques are being replaced by chromatographic methods (14), which provide the opportunity to fractionate samples without alteration or loss of material. Chromatographic schemes have been applied to the separation of both intact triglycerides (15) and derived fatty acid esters (16–18). More often, a preliminary separation of the intact triglycerides is followed by transesterification and chromatography of the fatty acid esters (19,20). Adsorption chromatography and reverse-phase partition chromatography are popular choices when combined with high-performance size-exclusion chromatography (21) and gas chromatography (22).

Our objective was the development of a similar analytical method capable of making detailed qualitative comparisons between different oils, including those that do not have the traditional glyceride backbone. The method would allow one-to-one comparisons of the major components, as well as the individual minor components present at low ppm levels. The approach was three-fold—first, oil samples were converted to the corresponding fatty acid methyl esters by transesterification. This step is essential for making comparisons between glyceride and non-glyceride oils, *i.e.*, soybean oil and olestra (a mixture of octa-, hepta- and hexaesters of sucrose, formed by reaction of sucrose with long-chain fatty acids). Second, the fatty esters were separated into fractions of differing polarity by adsorption chromatography and solid-phase extraction methods. Third, isolated fractions were profiled by capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC).

The work reported here details the first two parts of the analytical approach—methylation (Part I) and isolation (Part II) of the fatty acid methyl esters from heated soybean oil and heated olestra. The approach is, in part, a modification of the procedures used by Dobarganes (14), Ottaviani (17) and Perrin (18). As an important part of this work, we include the identification of the major components contained in each of the fractions. These identifications validate the separation scheme and serve as a useful reference for others attempting such separations. A third step features the chromatographic (GC and HPLC) finger-printing of the isolated fractions to allow detailed comparisons of the used frying oils (soybean oil and olestra). The results of these comparisons are provided in a companion publication (23).

EXPERIMENTAL PROCEDURES

Sample history. Refined, bleached and deodorized soybean oil was partially hydrogenated to an iodine value of 89. Olestra (76.3% octaester, 23.3% heptaester, 0.2% hexaester and 0.2% pentaester) was synthesized from sucrose and fatty acid methyl esters (derived from the soybean oil stock) as described by Volpenhein (24). The olestra and soybean oil were heated simultaneously in separate fryers to ensure equivalent heat and time exposure. Industrial food service fryers (8-kg capacity) were used, each containing 7.3 kg of frying oil at the start of each day. The average amount of oil in the fryers was 6.4 kg, as a result of oil removal by the fried food. Fresh oil was added at the end of each day to replenish what was removed. Prior to top-off, the weight of used oil in each fryer was made equal by removing oil from the fryer with the higher oil content. Peeled raw potatoes were hot-water blanched prior to frying and introduced into the fryers at a rate of 7.6 kg/h during the frying of 49 batches per day. The oils were heated to 185°C for 12 hours per day for seven days and the potatoes were fried during eight of these hours. After frying, samples of used oil, along with remains of the original oils, were stored in glass jars at 4°C. Samples were later fractionated according to the scheme provided in Figure 1.

Sample methylation. All samples were methylated by reacting 10 g of sample with 50 mL of a 0.13 M sodium

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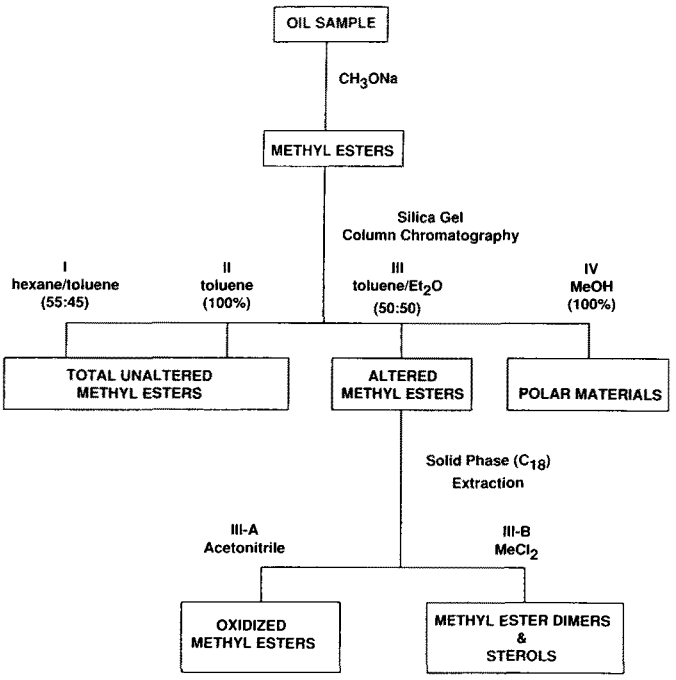


FIG. 1. Fractionation scheme used to separate heated oil fatty acid methyl esters into fractions of differing polarity.

methoxide in methanol solution with stirring in a 150-mL beaker. Samples were reacted for 25 min at 200–225°C, after which 25 mL of a saturated NaCl/HCl (1.3%) solution was added with stirring. Samples were removed from heat and mixed with 50 mL of n-hexane. The separated hexane layer was filtered through 5 g of sodium sulfate (anhydrous powder, E.M. Industries, Cherry Hill, NJ) and collected. Proton nuclear magnetic resonance (NMR) analysis of the filtrate was used to verify the completeness of the methylation. The filtrate was evaporated under dry nitrogen, yielding the methyl esters.

Silica gel column chromatography. Two grams of the methyl esters were loaded onto the top of a 15-mm (i.d.) glass column packed with 22.5 g of silica gel 60 (5% H_2O (E.M. Industries, Gibbstown, NJ) previously equilibrated with hexane/toluene (55:45) (UV grade, Burdick & Jackson, Muskegon, MI). Four fractions were eluted from the column in a stepwise fashion by using 150 mL of each of the solvents specified in Figure 1. Fractions were evaporated under dry nitrogen and weights were recorded (Table 1). Until additional analyses could be completed, Fractions I, II and III were redissolved in 5 mL of methylene chloride (MeCl), and Fraction IV was dissolved in methanol. All were stored at 4°C.

Solid-phase extraction. Fraction III was further separated by solid-phase extraction. A syringe extraction column was prepared by placing a C_{18} Sep-Pak (Waters and Associates, Milford, MA) cartridge on the Luer end of a 10-cc plastic syringe. The contents of a second Sep-Pak were placed into the syringe. This column was pre-washed with 5–10 mL of MeCl (UV grade, Burdick & Jackson), followed by 5–10 mL of acetonitrile (ACN) (UV grade, Burdick & Jackson). Solvents were forced through the column by a low-pressure flow of nitrogen. Fraction III methyl esters were dissolved in MeCl at a concentration not greater than 133 mg/mL. An aliquot (0.30 mL) of the dissolved sample was deposited onto the contents of two Sep-Paks in a 50-mL beaker. Following the evaporation of the MeCl, the deposited sample was mixed well with the remaining solid phase material and added to the top of the syringe column. The syringe column was eluted with 6 mL of ACN to yield Fraction IIIA, followed by 10 mL of MeCl to give Fraction IIIB. This procedure was repeated until all of Fraction III was fractionated. Collected fractions were evaporated under dry nitrogen and weights were recorded (Table 1). Fractions IIIA and IIIB were redissolved in 5 mL MeCl for storage at 4°C.

Capillary gas chromatography. Chromatograms were obtained with a Hewlett-Packard model 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) configured for capillary column operation with a split/splitless injector equipped with a splitless insert and flame ionization detector. Ultra high-purity helium (A-L Compressed Gases of Ohio, Cincinnati, OH) was used as the carrier for all gas chromatographic analyses with a linear velocity of 25 cm/min. Methyl pentadecanoate (Nu-Chek Prep Inc., Elysian, MN) was added as an internal standard to samples of Fractions I, II and IIIA. For Fractions I and II, 1 μL of sample was injected (splitless) with the following conditions: Column, DB-WAX (J&W Scientific, Folsom, CA), 15 m × 0.32 mm i.d., 0.25 micron film; injector temperature, 220°C; detector temperature, 250°C; column program, 50°C (2 min); 15°C/min to 170°C (1 min); 6°C/min to 200°C (1 min); 10°C/min to 220°C (20 min); vent flow, 100 mL/min; and injector vented after 1.5 min.

For Fraction IIIA, 1 μL of sample was injected (splitless) with the following conditions: Column, DB-WAX (J&W), 15 m × 0.32 mm i.d., 0.25 micron film; injector temperature, 220°C; detector temperature, 250°C; column program, 50°C (3 min); 20°C/min to 200°C (0 min); 2°C/min to 240°C (10 min); vent flow, 100 mL/min; and injector vented after 1.5 min.

Stearyl palmitate (Nu-Chek Prep Inc.) was added as an internal standard to Fraction IIIB samples. One μL of

TABLE 1

Fraction Weights from Separation of Fatty Acid Methyl Esters from Soybean Oil Before and After Heating

	I	II	IIIA	IIIB	IV	Recovery
Unheated oil (1.996 g)	1.810	0.124	0.020	0.009	0.012	
(% of total)	(90.7%)	(6.2%)	(1.0%)	(0.5%)	(0.6%)	98.9%
Heated oil (2.009 g)	1.721	0.114	0.062	0.047	0.017	
(% of total)	(85.7%)	(5.7%)	(3.1%)	(2.3%)	(0.8%)	97.6%

sample of Fraction IIIB was injected (in on-column mode) with the following conditions: Column, DB-5 (J&W), 30 m × 0.32 mm i.d., 0.10 micron film; detector temperature, 350°C; column program, 40°C (2 min); 25°C/min to 200°C (1 min); 5°C/min to 345°C (10 min); vent flow, 100 mL/min; and injector vented after 1.5 min. These GC conditions allowed for the observation of species below the 5 ppm level.

Capillary gas chromatography/mass spectrometry (GC/MS). Mass spectrometric data were collected on a Kratos MS-30 (Manchester, U.K.) interfaced to a Siemens Sichromat 2, two-dimensional gas chromatograph (E.S. Industries, Marlton, NJ). Splitless sample introductions of Fraction IIIA were made with one-dimensional separations performed according to the following conditions: Column, DB-WAX (J&W), 30 m × 0.32 mm i.d., 0.25 micron film; injector temperature, 220°C; detector temperature, 250°C; column program, 50°C (3 min); 20°C/min to 220°C (0 min); 3°C/min to 240°C (10 min); column head pressure, 8 psi; vent flow, 50 mL/min; and injector vented after 0.6 min.

Electron ionization (70 eV) and chemical ionization (isobutane and ammonia) mass spectra were obtained at a scan rate of 1 s/decade and a resolution of 1000 (10% valley). Source temperature was 250°C. Isobutane was admitted to a manifold pressure of 160 millitorr, which results in a 57:43 *m/z* ratio of approximately 3:1. Ammonia was admitted to a manifold pressure of 370 millitorr, which results in a 18:35 *m/z* ratio of approximately 3:1.

Capillary gas chromatography/Fourier transform-infrared spectroscopy (GC/FTIR). GC/FTIR data were obtained from a Digilab FTS-15E FTIR spectrometer (Cambridge, MA) connected to a Hewlett-Packard 5880A GC *via* a Model GC/C 32 light-pipe interface (Bio-Rad, Cambridge, MA). Fraction IIIA sample introduction was made in a splitless mode with the following chromatographic conditions: Column, DB-WAX (J&W), 30 m × 0.32 mm i.d., 0.5 micron film; injector temperature, 220°C; detector temperature, 250°C; column program, 50°C (3 min); 20°C/min to 220°C (0 min); 4°C/min to 240°C (10 min); vent flow, 100 mL/min; injector vented after 1.5 min; and light-pipe transfer line temperature, 260°C. All FTIR data were collected at an interval of 4 scans per s and 8 cm^{-1} resolution, with each spectrum representing the co-addition of 12 or more scans.

High-performance liquid chromatography (HPLC). Chromatograms were obtained with a Hewlett-Packard 1090L liquid chromatograph equipped with two Ultrasphere columns (ODS, 5 μm, 150 mm × 4.6 mm) (Beckman, San Ramon, CA) connected in series. Column temperature was maintained at 40°C. An isocratic separation of Fraction IV was achieved at a flow rate of 1 mL/min and a mobile phase composition of acetone/methanol (0.01% acetic acid; 20:80, vol/vol). Column eluent was detected with an ACS Model 750:14 evaporative light scattering detector (Polymer Laboratories, Stow, OH) operated at 40°C, and a nebulizer pressure of 40 psi nitrogen.

Chromatographic data collection, integration and plotting. Analog data output from gas and liquid chromatographic detectors was transmitted through Hewlett-Packard 18542A Analog to Digital converters to a Hewlett-Packard 1000 computer, where data were integrated by means of Hewlett-Packard Laboratory Automation System (LAS rev.D.01) software. CPLOT/3350 (Hewlett-Packard) software was used to format (overlay and scale) chromatographic plots.

RESULTS AND DISCUSSION

The weights of material for each fraction are displayed in Table 1, along with the recoveries from the triglyceride (only) samples. Similar results were obtained from the olestra samples, the details of which can be found in the second half of this work (23). Upon heating, Fraction I and II weights decrease slightly, Fraction IIIA and IIIB weights increase dramatically, and Fraction IV weight increases significantly. Fractions I and II are primarily normal, unaltered methyl esters as described below. Virtually all of the new materials formed upon heating appear in Fractions IIIA and IIIB, consistent with the functional nature of oxidation products reported in the literature. Consequently, most of the results and discussion of this work focus on Fractions IIIA and IIIB.

Fractions I and II. The gas chromatographic profile of Fraction I from the heated triglycerides is shown in Figure 2. The Fraction I composition is similar to that of the unaltered fatty acid methyl esters that make up the initial triglycerides. A Fraction II profile is not shown because it contains the same components as Fraction I. Together, Fractions I and II comprise 92% of the total methyl esters from the heated triglyceride sample, a good estimate of the percentage of unaltered methyl esters.

The abundance of unaltered methyl esters in Fractions I and II make it difficult to observe minor components in these fractions. Known altered components expected in Fractions I and II from heated oils include cyclic fatty acid monomers, which are measured by a separate procedure (25). Volatile hydrocarbons (*e.g.*, pentane) and aldehydes (*e.g.*, hexanal) are known oxidation products expected in Fraction I, but no effort was made to retain these during solvent removal. Fraction I also contains such naturally occurring hydrocarbons as squalene and dehydrated sterols, but these compounds were not detected in the present analyses due to the overwhelming abundance of unaltered methyl esters in Fraction I.

Fraction IIIA. As indicated in Table 1, Fraction III accounts for 5.4% of the methyl esters in heated triglycerides (as compared to 1.5% in unheated triglycerides). The majority of these altered methyl esters are in Fraction IIIA, the gas chromatographic profile of which is displayed in Figure 3. Four major classes of altered methyl esters are present—aldehydes, epoxides (or oxiranes), ketones and alcohols. Examples of vapor-phase infrared and mass spectra from each class are discussed below and were obtained from peaks that are major components in both heated triglyceride and heated olestra samples.

Aldehydes. The most abundant aldehyde observed is C9:0 aldehyde methyl ester (or methyl 9-oxononanoate), labeled as peak A in Figure 3. The vapor-phase infrared and electron ionization mass spectra are displayed in Figure 4. Isobutane and ammonia chemical ionization (CI) mass spectra are tabulated in Table 2. The CI data clearly define the molecular weight to be 186. The infrared spectrum is conclusive for the aldehyde functionality due to the specific aldehydic C-H stretch at 2712 cm^{-1}). The ester (1755–1765 cm^{-1}) and aldehyde (1745–1750 cm^{-1}) C=O stretches are not resolved (26), and the remainder of the infrared (IR) spectrum is typical of methyl esters.

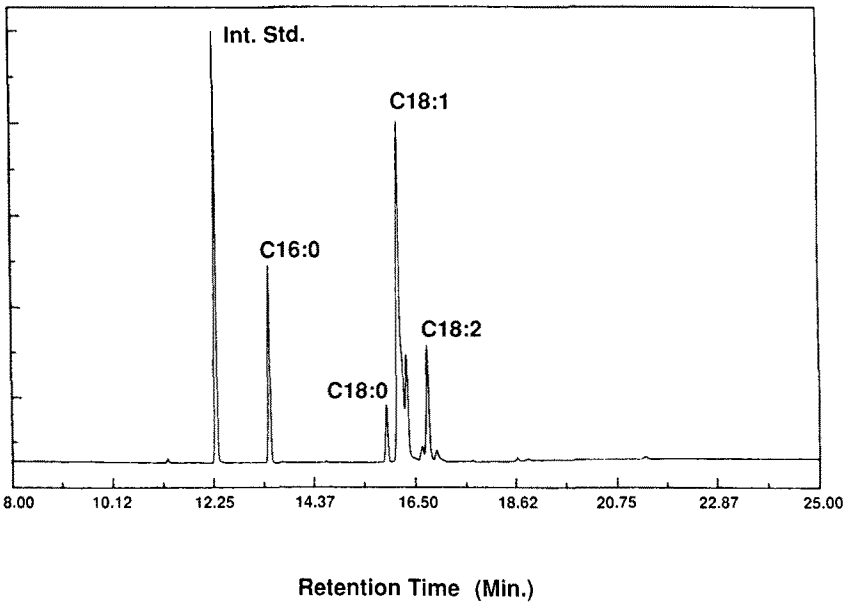


FIG. 2. GC chromatogram of Fraction I containing unaltered fatty acid methyl esters.

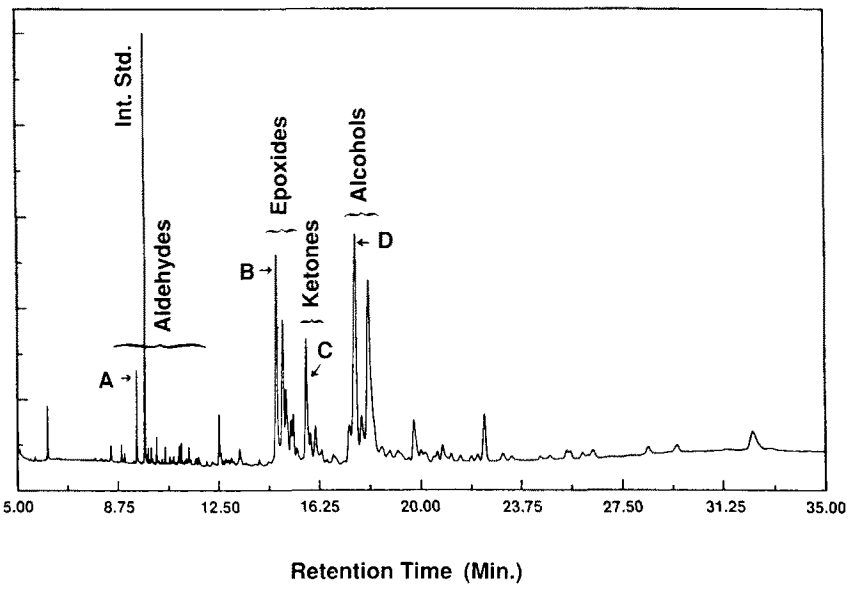


FIG. 3. GC chromatogram of Fraction IIIA containing altered fatty acid methyl esters.

The electron ionization (EI) mass spectrum reveals fragments characteristic of straight-chain methyl esters (74, 87, 59) and also contains a small M-28 ion (*m/z* 158) often observed for aldehydes (27). The large ($MH^+ - CH_3OH$) peak at *m/z* 155 in the isobutane CI spectrum (Table 2) is typical of the aldehyde methyl esters observed in these samples. In the case of C=C unsaturated aldehyde methyl esters, the infrared spectra provide additional information. Conjugation of the carbon-carbon and the carbon-oxygen double bonds removes the degeneracy of the carbonyl stretches. Therefore, the presence of an aldehydic C-H stretch at 2712 cm^{-1} and carbonyl stretches at 1755 cm^{-1} (ester) and 1715 cm^{-1} (conjugated aldehyde or ketone) specifies the conjugated aldehyde, thus pinpointing the locations of the carbonyl group and the carbon-carbon double bond (26). Using this combination of GC/IR and GC/MS data, C10:1, C11:1 and C12:1 aldehyde methyl esters were identified in both heated triglyceride and heated olestra.

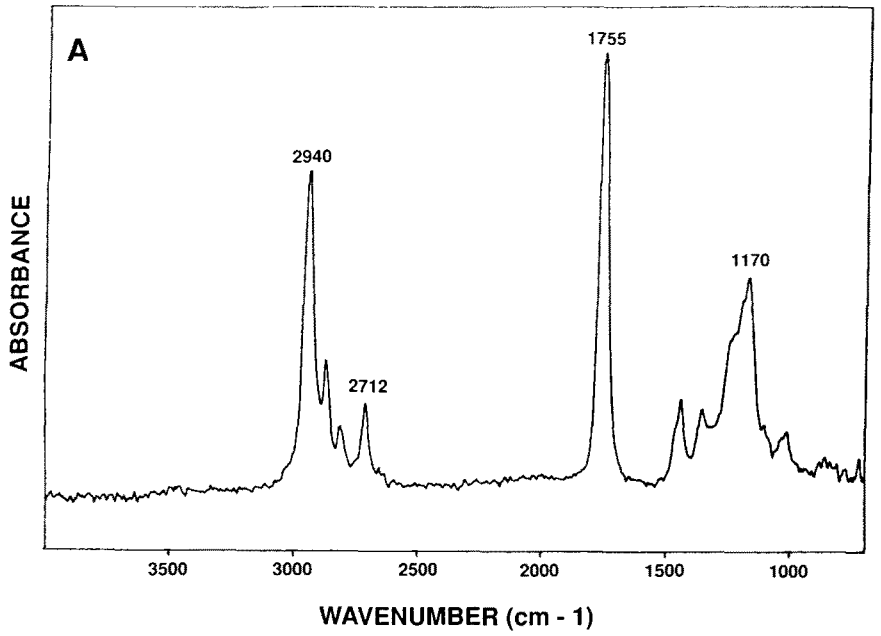
Epoxides. Vapor-phase infrared and electron ionization mass spectra for the most abundant epoxide (methyl 9,10-epoxyoctadecanoate) are shown in Figure 5. This component is labeled as peak B in Figure 3. Isobutane and

TABLE 2

Isobutane and Ammonia Chemical Ionization Mass Spectra of Identified Components from Fraction IIIA

Compound	MW	Isobutane spectrum *m/z* (rel. int.)	Ammonia spectrum *m/z* (rel. int.)
Methyl 9-oxononanoate	186	188 (10), 187 (100), 156 (8), 155 (80)	205 (10), 204 (100), 187 (3)
Methyl 9,10-epoxyoctadecanoate	312	313 (40), 296 (20), 295 (100), 281 (64) 263 (48), 199 (15), 187 (18), 185 (15) 171 (18)	331 (20), 330 (100), 313 (18) 312 (14), 295 (18)
Methyl oxooctadecanoate	312	314 (23), 313 (100), 282 (7), 281 (37)	331 (20), 330 (100), 313 (13)
Methyl hydroxyoctadecanoate[a]	314	313 (2), 298 (21), 297 (100), 295 (9) 265 (3)	333 (19), 332 (87), 330 (62) 315 (18), 314 (65), 298 (20) 297 (100), 295 (20)

[a]There is some hydroxy-C18:1 contribution to these spectra, due to co-elution.



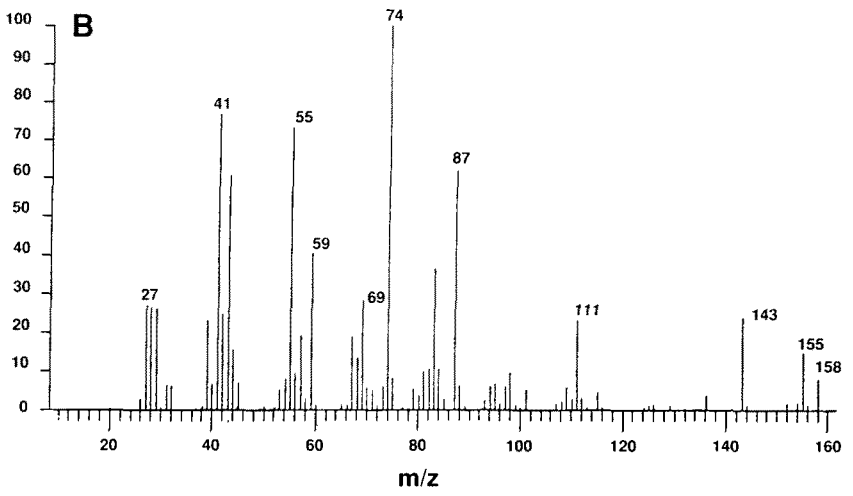


FIG. 4. Vapor-phase infrared (A) and electron ionization mass spectrum (B) of methyl 9-oxononanoate.

ammonia CI data are tabulated in Table 2. The infrared spectrum (Fig. 5A) has little to distinguish it from normal methyl esters, except for unique bands in the 840–910 cm^{-1} region, which are consistent with absorptions attributed to epoxides (28). The reference EI spectrum (not shown) of methyl 9,10-epoxyoctadecanoate was the best match retrieved for Figure 5B from a search of the National Institute of Standards and Technology (NIST) Library (Gaithersburg, MD). CI data confirm the molecular weight of 312. The isobutane CI spectrum reveals a characteristic pattern of M + 1 − 18, M + 1 − 32 and M + 1 − 18 − 32 due to losses of water, methanol and both of these, respectively, from the protonated molecule. This pattern is consistent with the pattern reported for epoxides (29).

Ketones. Figure 6 displays the vapor-phase infrared and EI mass spectra of the most abundant ketone (C18:0 ketone methyl ester or methyl oxooctadecanoate), labeled as peak C in Figure 3. Isobutane and ammonia CI data are tabulated in Table 2. The infrared spectrum, Figure 6A, is conclusive for the ketone functionality, providing a carbonyl absorption (1728 cm^{-1}) distinct from the ester carbonyl (1759 cm^{-1}) without the corresponding aldehydic C-H stretch (2712 cm^{-1}). A search of the EI mass spectrum in Figure 6B against the NIST Library retrieved 9-oxooctadecanoic acid methyl ester as the best match. CI data confirm the molecular weight of 312, and the CI fragmentation matches that described for keto methyl esters (29). Only the exact location of the keto

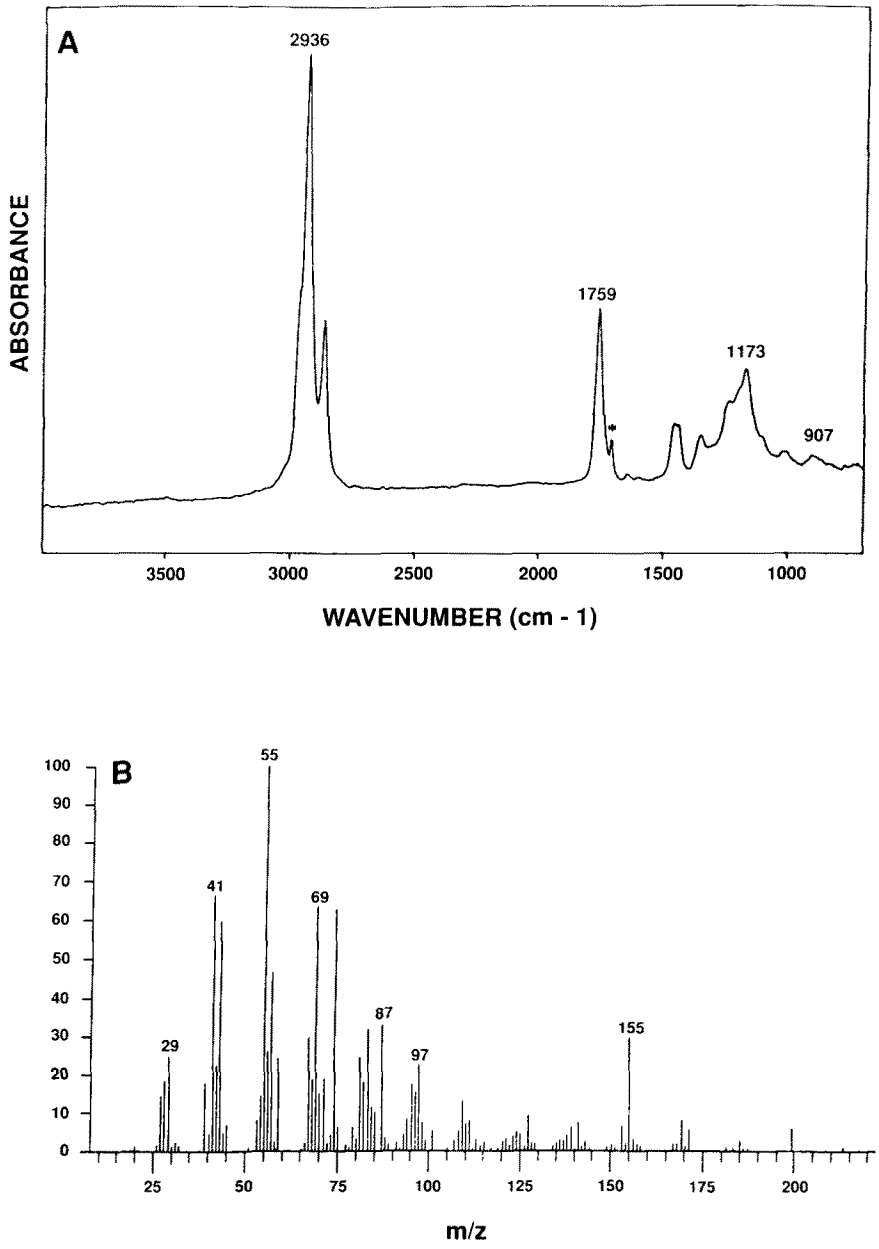


FIG. 5. Vapor-phase infrared (A) and electron ionization mass spectrum (B) of methyl 9,10-epoxyoctadecanoate. (*) Possible co-elution with ketone methyl ester.

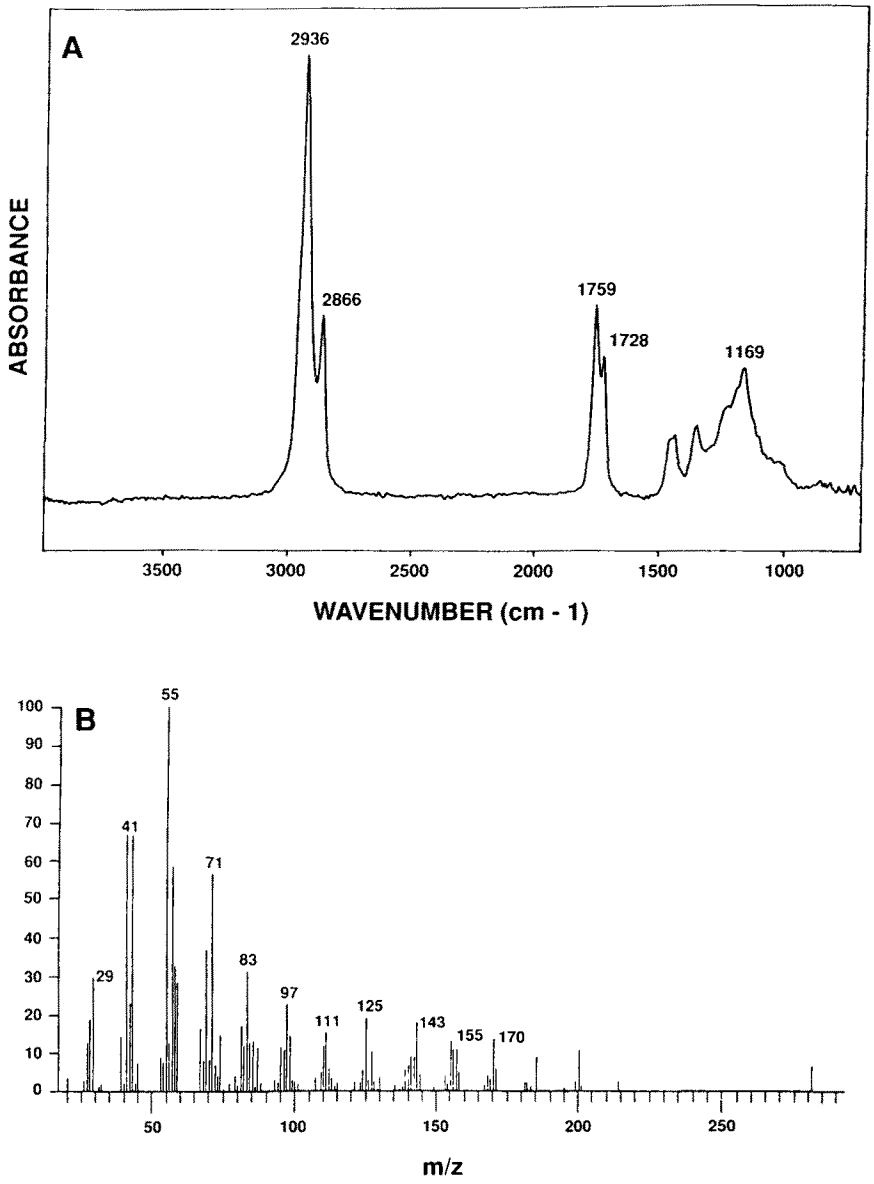


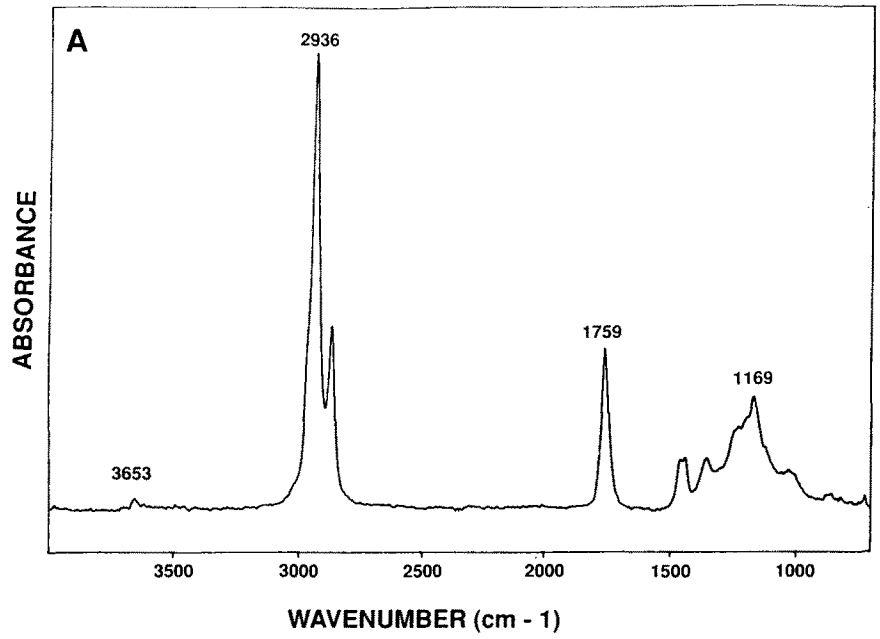
FIG. 6. Vapor-phase infrared (A) and electron ionization mass spectrum (B) of methyl oxooctadecanoate.

group is in question, since EI fragmentation does not specify the location clearly.

Alcohols. The vapor-phase infrared and EI mass spectra of C18:0 alcohol methyl ester (or methyl hydroxyoctadecanoate) are shown in Figure 7. This component is labeled as peak D in Figure 3. Isobutane and ammonia CI data are tabulated in Table 2. The vapor-phase infrared spectrum (Fig. 7A) reveals a small but significant O-H stretch (3653 cm^{-1}) in addition to absorptions typical of methyl esters in general. Ammonia CI data are the most useful for molecular weight (314) verification in this case, providing ions at M + 18, M + 18 − 18 and M + 1 − 18 due to ammonium cationization, ammonium cationization followed by loss of water and protonation followed by loss of water, respectively. The isobutane CI spectra reveal only water loss fragments from the protonated molecule. These CI spectra are consistent with those reported for hydroxy fatty acid methyl esters (29).

Gas chromatographic profiles of Fraction IIIA also were obtained from a low-polarity column (DB-5). Much greater peak overlap was observed with this column, and only one new set of components was revealed. These were monoglycerides, resulting from incomplete methylation of the triglyceride sample. These monoglycerides, which do not elute from the polar GC column under the conditions used, also spill over into Fraction IV, as noted below.

Normal and reverse-phase HPLC profiles of Fraction IIIA also were obtained to assure that major non-volatile species were not missed. Because of the complexity of Fraction IIIA and the lower sensitivity of the liquid



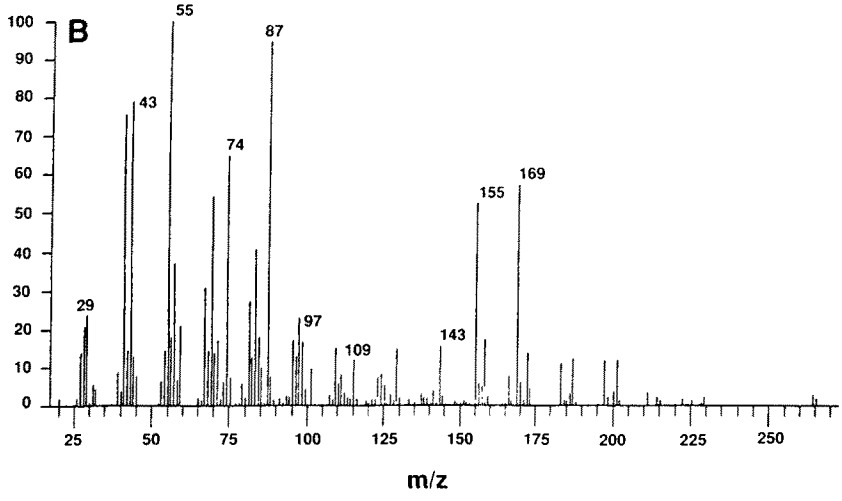


FIG. 7. Vapor-phase infrared (A) and electron ionization mass spectrum (B) of methyl hydroxyoctadecanoate.

chromatographic profiles, the HPLC approaches used to detect minor, altered methyl esters yielded no additional components to those found by gas chromatography.

Fraction IIIB. Fraction IIIB also contains a significant portion of the altered methyl esters generated upon heating. Since this fraction contains high-boiling components, a GC column stable to high temperatures was used. The gas chromatographic profile of this fraction from heated triglycerides is shown in Figure 8. There are two major classes of compounds in this fraction as determined by GC/MS analysis. In the 14–16 min elution region are the three phytosterols (campesterol, stigmasterol and sitosterol) prevalent in soybean oil. Dimer fatty acid methyl esters (30) elute in the 21–27 min region. Mass spectra of dimer methyl esters from a similar sample have been presented previously (31). The earliest-eluting (6–10 min) components are unaltered fatty acid methyl esters (possible column breakthrough from Fraction II), and some of the same oxidized fatty acid methyl esters (FAME) observed in Fraction IIIA. These may result from incomplete separation in the Sep-Pak procedure, or they may result from thermal decomposition of hydroperoxides and peroxides upon injection into the hot injection port. Finally, the peak eluting just after the dimer methyl esters is a diglyceride (primarily diolein) resulting from incomplete methylation.

That the major components of Fraction IIIB are sterols and dimer methyl esters was confirmed by direct-probe ammonia chemical ionization mass spectrometry. The

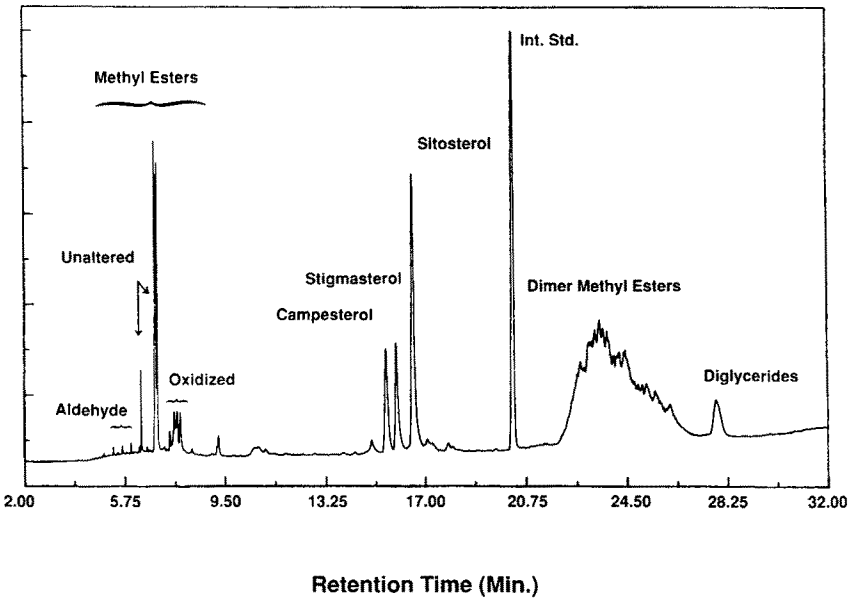


FIG. 8. GC chromatogram of Fraction IIIB containing dimer methyl esters and phytosterols.

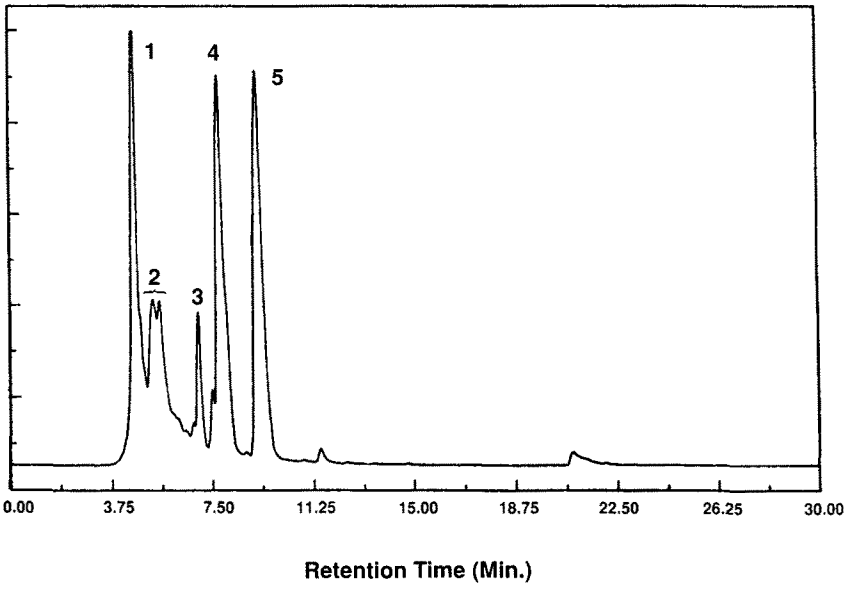


FIG. 9. HPLC chromatogram of Fraction IV containing highly polar altered fatty acid methyl esters.

above data show that the major components of this fraction can be eluted from the gas chromatograph, but the resolution of these complex high-boiling species is not as great as for Fraction IIIA. As with Fraction IIIA, normal and reverse-phase HPLC profiles of Fraction IIIB also were obtained. For this fraction, HPLC profiles were nearly as detailed as the GC profiles, but no additional components were identified. All of the compounds identified above have been reported previously as autoxidation products of methyl oleate or methyl linoleate (32).

Fraction IV. Fraction IV represents only 0.8% of the heated triglyceride sample (0.6% of the unheated sample). Because of the high polarity of this fraction, profiling was accomplished by HPLC rather than by GC. The HPLC profile for heated triglycerides is displayed in Figure 9. Five fractions, as indicated in the Figure, were collected and analyzed by direct exposure probe mass spectrometry. Peaks 1 and 5 are largely oxidized di- and triglycerides, the result of incomplete methylation. Peaks 3 and 4 are monoglycerides and also result from incomplete

methylation of the triglycerides. Only region 2 appears to be due to altered methyl esters. These peaks yield a chemical ionization mass spectrum that strongly resembles the spectrum of methyl 9-hydroperoxy-10,12-octadecadienoate reported by Plattner *et al.* (29). Extensive peak overlap prevents more definitive identifications of Fraction IV components.

The fractionation scheme reported here allows the examination of all the FAMEs from heated fry oils, directly, without loss and with minimal distortion of the sample. The typical classes of altered FAMEs previously reported from other schemes were accounted for by this scheme. Such separation methodology, coupled with the use of high-resolution chromatographic profiles of the five individual fractions, can serve as the initial stage of a comprehensive comparison of heated oil samples and is necessary for comparing traditional triglyceride oils with new, alternative fatty acid-based oils.

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