Isolation and Characterization of Dimers Formed in Used Soybean Oil

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Dimers were isolated from two samples of partially hydrogenated soybean oil which had been used for frying. They were then separated into their component structures using an LC-18 octadecyl bonded, HPLC column with acetone-acetonitrile (1:1, v/v) as the mobile phase with refractometry as the mode of detection and identified by gas chromatography-mass spectrometry. Evidence is presented for the presence of the monohydroxy, dihydroxy, and keto groups in the dimer of linoleic acid as well as the dehydrodimer, the bicyclic, the tricyclic, and the thermal dimer of methyl linoleate and the dehydrodimer of methyl oleate.

The isolation and structure elucidation of dimers formed as the result of thermal-oxidation of fats during deep frying has been of great interest. However, the compositional determination of this fraction from used or heated fats and oils has been neglected because of the difficulties encountered during the isolation of dimers from heated fats and the complexity of the structures present within this fraction.

The presence of polar dimers in thermally oxidized corn oil at 200°C was reported by Perkins and Kummerow (1). Dimers and higher polymers isolated had a molecular weight range of 692 to 1600 daltons; they were non-cyclic, of high oxygen content, and they contained unsaturation difficult to remove by hydrogenation. The oxygen present in the fractions was shown to be in the form of hydroxyl and carboxyl groups. Similarly, Sahasrabudhe and Farn (2) presented results of the effect of heating corn oil in air at 200°C. Dimeric and polmeric products were isolated from the heated oil and evidence was presented for the presence of hydroxy acids in the saponified polymeric fractions.

Firestone *et al.* (3) heated cottonseed oil at 205 and 225°C in the presence of air. The dimers and higher polymers that were isolated contained moderate amounts of carbonyl and hydroxyl groups and unsaturation difficult to remove by hydrogenation. However, the dimers—after bromination-dehydrobromination and oxidation—absorbed in the ultraviolet region at 250–260 and 270–280 nm, indicating the presence of cyclic structures. In addition, Barrett and Henry (4) presented chromatographic and spectral evidence for the presence of dimers in cottonseed oil which had been used for frying.

Ohfuji and Kaneda (5) oxidized soybean oil at 295°C for 12 hours in the presence of nitrogen or air. The dimeric fraction had a molecular weight of 586 daltons and contained several functional groups such as carbonyl, hydroxyl, and epoxide groups. Treatment with sodium borohydride and hydroiodic acid revealed no peroxidic or ether linkages. The content of conjugated dienes was low, but the strong UV absorption at 223 nm suggested ketones conjugated with double bonds. Furthermore, this dimeric fraction was proven to be very toxic to mice.

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Zeman and Scharmann (6) reported the presence of 0.3% non-polar dimers composed of diunsaturated bicyclic and tetraunsaturated acyclic structures in peanut oil subjected to thermal and oxidative action. Perrin *et al.* (7) analyzed samples from two different peanut and sunflower oils oxidized by deep fat frying to a stable foam formation. They reported the presence of dimers at levels between 12.1 and 12.9% of the oxidized mixtures. Furthermore, peanut oil oxidized by deep fat frying before and after stable foam formation yielded 7.8 and 14% dimers, respectively. Gere *et al.* (8,9) reported non-polar and polar dimeric triglycerides in sunflower oil used in deep fat frying.

Cypranycz *et al.* (10) reported the presence of dimeric triglycerides in various butterfat samples oxidized at 185°C in the presence of air for 8 and 16 hours. Non-polar and polar dimeric triglycerides were found in lard samples used in deep fat frying (8,9).

The results of dimer formation in ten different oils oxidized to the level of stable foam formation were presented by Perrin *et al.* (7). The dimer content was found to be between 1.6 to 17.5% of the nonvolatile decomposition products with 1.6% for the beef tallow and 17.5% for the soybean oil sample, respectively.

Furthermore, thermal as well as oxidative dimers have also been isolated and characterized from pure fatty acid and triglycerides oxidized under simulated deep fat frying conditions. These model systems have been employed in order to simplify and control the various parameters affecting the thermal-oxidative reactions and to facilitate the structure elucidation of the decomposition products.

Michael *et al.* (11) heated methyl linoleate diluted with an equal weight of methyl laurate at 200°C for 200 hours in the presence of air. The dimer was isolated from the reaction mixture and further separated into nonpolar and polar fractions. Analytical data for the non-polar dimers were consistent with the cyclic Diels-Alder structure, whereas the polar dimer material was non-cyclic, containing hydroxyl, peroxy, and/or carbonyl groups. Perkins and Wantland (12) subjected pure 1-linoleyl-2-3-distearin to thermal oxidation at 200°C and presented evidence for the presence of cyclic and noncyclic dimeric species in the reaction mixture.

Chang *et al.* (13) presented studies on the nonvolatile decomposition products from pure trilinolein, triolein, and tristearin produced under simulated deep fat frying conditions at 185°C for 74 hours. Chromatographic, chemical, and spectrometric analysis indicated the presence of dimers in all three oxidized triglyceride mixtures. A cyclic carbon to carbon linked dimer and a noncyclic dimer that was formed through carbon to carbon linkage and contained two hydroxyl groups per molecule were identified from the oxidized trilinolein mixture. The cyclic and noncyclic dimer presented 4.9 and 2.8% of the treated trilinolein, respectively (14). Triolein also yielded two different noncyclic dimers joined by carbon to carbon linkages. Each dimer constituted 1.36% of the treated triolein, one was determined as the dimer of methl oleate and the other as the dimer of methyl oleate with one

carbonyl group per molecule. Finally, tristearin yielded noncyclic dimers joined by carbon to carbon linkages and constituted 0.7% of the oxidized tristearin.

Developments in the chromatography of dimers, as well as the synthesis and characterization of model dimers with various structural features as previously described (15) allowed the separation of dimeric fractions into their component types. In the present study, these fractions from two samples of soybean oil used for frying were isolated and fractionated into their component dimers which were structurally characterized by GC-MS.

EXPERIMENTAL

Chromatographic methods. The High Performance Size Exclusion of Gel Permeation Chromatographic (HPSEC) systems used for the fractionation of oxidized oils were described earlier (16). HPSEC analyses were carried out on one Lichrogel PS_4 and one Lichrogel PS_1 column, 25 × 0.7 cm i.d., connected in series (E.M. Science, Gibbstown, NJ). SEC analyses were performed on two glass columns, 109 cm × 12.5 mm i.d., connected in series, and packed with Bio-beads SX-2 (Bio-Rad Laboratories, Richmond, CA). Toluene was used in both systems as the eluent at a flow rate of 0.5 and 1 ml/min for HPSEC and SEC, respectively. Refractometry was the mode of detection. Sample concentration was 25 mg/ml for HPSEC and 100 μl was the injection volume for SEC. Calculation of correction factors and quantitation were performed as described earlier (17).

Packed and capillary GLC were carried out as mentioned (17). Packed column GLC was performed on a well conditioned 3% OV-17, 6 ft × 2 mm i.d. glass column. The chromatographic conditions were: Detection (FID) and injector at 360°C, oven programmed from 300°C to 330°C at 4°C/min, followed by 5 min at 350°C, and nitrogen carrier gas at 35 ml/min and sample concentration was 5–10 mg/ml. Capillary GLC was carried out on an HP-5 column packed with crosslinked 5% phenyl methyl silicone (Hewlett Packard, Avondale, PA), 25 m × 0.31 mm i.d., and 0.17 μm film thickness. Chromatographic conditions were: Oven temperature 300°C with a temperature rate 1°C/min to final temperature 310° for 10 min, injector temperature 330°C, detector (FID) temperature 360°C, carrier gas H_2 at 30 ml/min, split ratio 1:100 and sample concentration 0.1–0.5 mg/ml. Data is expressed as retention time (min) (RT), or as relative retention time (min) RRT.

HPLC was performed as previously described (15). Two different systems were employed. In System I, analysis was carried out on an LC-18 column, 25 cm × 4.6 mm i.d., dp = 2 μm (Supelco, Inc., Bellefonte, PA) with acetone-acetonitrile (1:1, v/v) as the mobile phase at a flow rate of 0.5 ml/min, with refractive index as the mode of detection and sample concentration 25 mg/ml. System II employed an LC-18, 15 cm × 4.6 mm i.d., dp = 2 μm (Supelco, Inc., Bellefonte, PA) column with acetonitrile as the mobile phase (Spectro, E.M. Science, Cherry Hill, NJ) at a flow rate of 1 ml/min ultraviolet detection at 205 nm, and sample concentration of 1–5 mg/ml.

Preparation of samples. Both oxidized samples were prepared from partially hydrogenated soybean oil (PHSO). Rojo and Perkins (18) heated PHSO under simulated deep fat frying conditions at 195 ± 5°C for 80 hours, 8 hours/day. Moistened cotton balls were fried every 30 minutes (oxidized Sample A). Pinter and Perkins (19) heated PHSO at 180 ± 5°C for 72 hours, 8 hours/day. Unpeeled potato slices were fried every 30 minutes (oxidized Sample B).

Samples were analyzed as methyl esters prepared in accordance with AOCS Official Method Ce 1-62 (20).

TMS derivatives of the polar isolated dimeric fractions from both samples were prepared as following: 400 μl of pyridine (A.C.S. grade, Mallinckrodt, Paris, KY) and 100 μl of bis-(Trimethyl)-Trifluoroacetamide (Supelco Inc., Bellefonte, PA) were added to 1–5 mg of sample in a 1 ml screw capped glass vial fitted with a teflon lined septum (Supelco, Inc., Bellefonte, PA). The vial was then capped, shaken, and heated for 20 minutes at 60°C. The solution of the TMS derivatives was used within four hours.

Hydrogenation. The Parr apparatus (Parr Instruments, Moline, IL) was used for the hydrogenation of the oxidized oil samples and/or sterically hindered samples. Ethyl acetate was employed as solvent, and platinum oxide was added at about 100 mg, regardless of the sample size (1.5–5.5 mg). Small amounts (1–5 mg) of dimeric methyl esters were catalytically hydrogenated using a microhydrogenator apparatus (Supelco, Inc., Bellefonte, PA).

Gas chromatography-mass spectrometry. GC-MS analyses were performed on a Hewlett-Packard Model 5985B GC-MS (Hewlett-Packard Co., Avondale, PA). The mass analyzer was a quadruple type and the electron impact ion (EI) source set at 70 eV. The chemical ionization (CI) gas used was methane. The packed GLC column and conditions employed are given above. Copies of individual mass spectra are available from the authors.

RESULTS AND DISCUSSION

The HPSEC chromatograms of the methyl esters from fresh and used soybean oil samples that are identified in Figure 1 as B and C are presented. The composition of the thermally oxidized oil in sample B was 90.17% monomer, 6.63% dimer, and 3.16% trimer. Sample C was considerably more polymerized with 77.82% monomer, 15.23 dimer, and 6.95 trimer. Quantitation data were determined by HPSEC and correlated well with data obtained from either GLC analysis or gravimetrically by SEC. Further information on the characteristics of the oils before and after oxidation has been presented by Rojo and Perkins (18), and Pinter and Perkins (19).

The dimeric fractions of used oils termed A and B were isolated by SEC and analyzed by the most efficient packed (Fig. 2) and capillary (Fig. 3) systems for dimer analysis developed previously for a series of synthetic dimers used to prepare a mixture of known composition. The components in the mixture were: the thermal, dehydro, and becyclic dimers of methyl linoleate (Me-Ln), the dehydro dimer of methyl oleate and the tetrahydroxy, dihydroxy, and diketo dimers of methyl stearate (15).

An effort was made to identify the various peaks representing dimers in the capillary GLC separations of the dimeric fractions A and B from a used fat. This was done by comparing the relative retention times (RRT) or equivalent hydrocarbon lengths of the dimeric components with those obtained for the various synthetic by dimers used as standards and analyzed under chromatographic

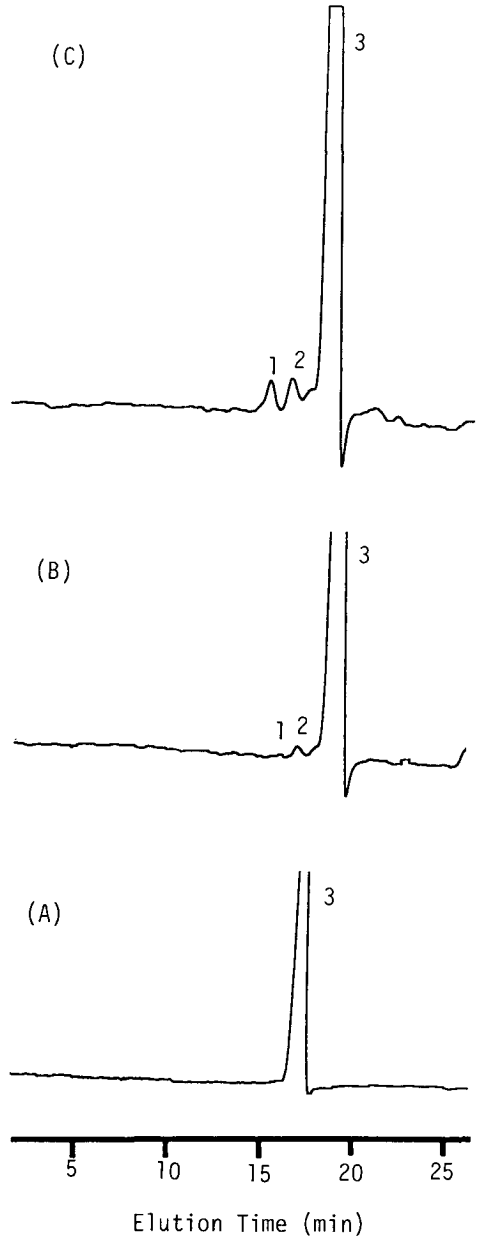


FIG. 1. HPSEC chromatograms of fresh (A) and oxidized oil samples B and C as FAME. Peak 1, 2, 3 are trimer, dimer, and monomer, respectively. Conditions : Column—series connected Lichrogel PS_4 and PS_1, 25 cm × 0.7 cm i.d., 5 μ particle size; eluant—toluene at 0.5 ml/min; sample concentration—25 mg/ml in toluene, 20 μl injection volume, refractive index detection.

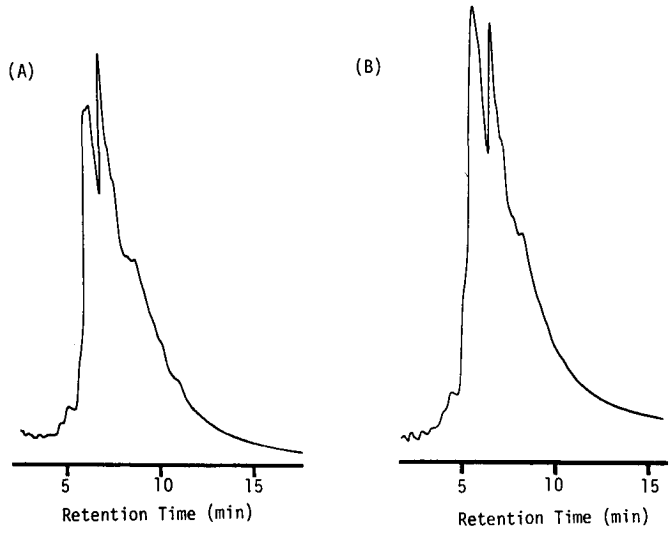


FIG. 2. GLC chromatograms of dimer fraction from oxidized oils A and B (labeled B and C in Fig. 1). Conditions: column—3% OV-17 (phenyl methyl silicone, 50% phenyl) on 80–100 mesh, Supelcoport, 6 ft × 2 mm i.d., glass. Initial temp. 300°, final temp. 330°, programmed at 4°/min with a 20 min hold, injector—360°, detector—360°, carrier gas—N_2 at 35 ml/min, injection 5–10 μg in 1–2 μl solvent, dtector—FID, and electronic integrator.

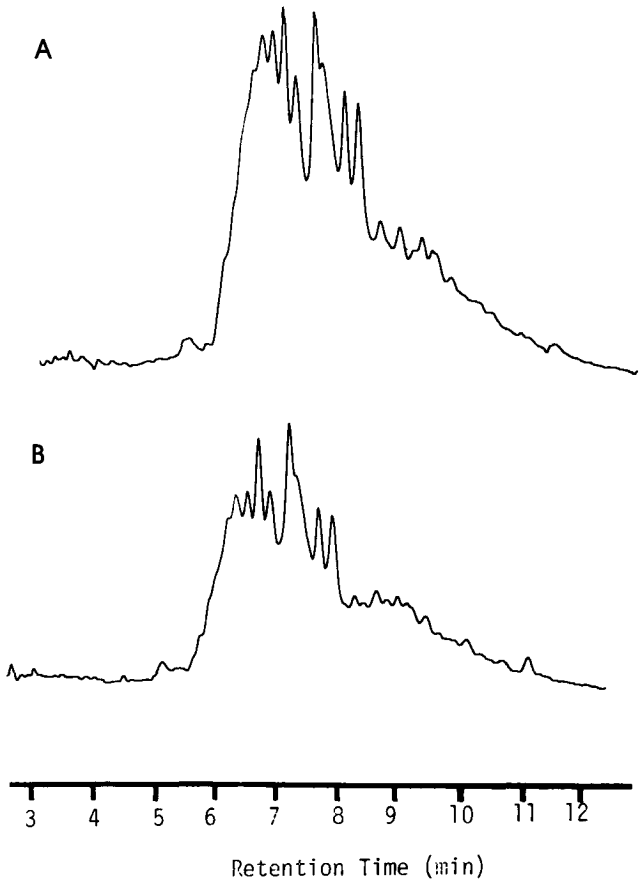


FIG. 3. Capillary GLC chromatograms of oxidized oil samples A and B. Conditions: column—25 mm × 0.31 i.d., fused silica. HP-5 (cross lined 5% phenyl methyl silicone), film thickness—0.17 μm, init. temp.—300°, final temp. 310°, program rate—1°/min with a 10 min hold, injector—330°, detector—360°, 1 μl inj. at 0.1–0.5 mg/ml conc. with a split ratio of 1:100, carrier gas—H_2 at 7.5 psig pressure.

conditions previously described (15). This proved to be a difficult task since several isomers are present and there is an overlapping from isomers of the various dimer types. Comparison of the retention data of the components from dimeric fractions A and B to those of the standard dimers by GC-MS analysis of both dimeric samples and their hydrogenated forms indicates the presence of different non-polar, as well as polar dimers, containing hydroxy and keto groups. However, tentative identification of the various peaks was not possible because of the separation achieved here.

HPLC provided the best means to analyze the various dimers within the dimeric fractions A and B. In

Figures 4-6, the HPLC separation of dimeric fractions of samples A and B with solvent systems I and II are presented. Comparison of the retention data of the dimers of A and B with those of the standard dimers analyzed under the same conditions indicated the presence of nonpolar as well as hydroxy and keto dimers. Fractions were collected as shown in Figures 4 and 5 from both dimeric samples with the use of system I and then subsequently analyzed by GC-MS. The HPLC curves shown in Figure 6, results from a solvent change to acetonitrile so that UV detection can be used, do not indicate improved separation. The peaks were more diffuse since a lower polarity eluant was employed. This system was not further investigated.

GC analysis of fraction I of dimeric sample A (Fig. 4) gave one broad peak which eluted in the area of

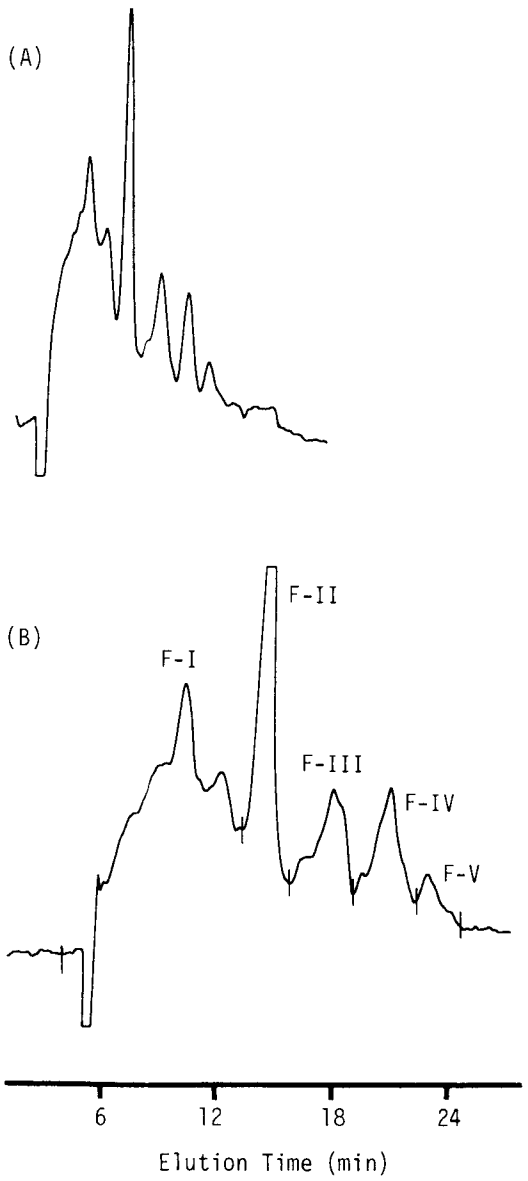


FIG. 4. HPLC chromatogram of dimers from oxidized oil sample A. Conditions: column—LC-18, 5 μ, 25 cm × 4.6 mm i.d., eluant—acetone/acetonitrile (1:1, v/v), detector—refractometry flow rate, A—1 ml/min, B—0.5 ml/min.

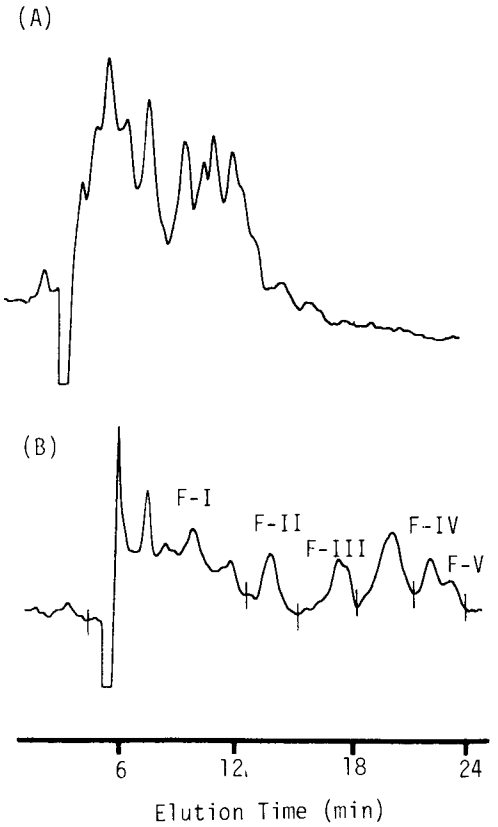


FIG. 5. HPLC chromatogram of dimers of oxidized oil sample B. Conditions: column—LC-18, 5 μ, 25 cm × 4.6 mm i.d., eluant—acetone/acetonitrile (1:1, v/v), detector—refractometry flow rate, A—1 ml/min, B—0.5 ml/min.

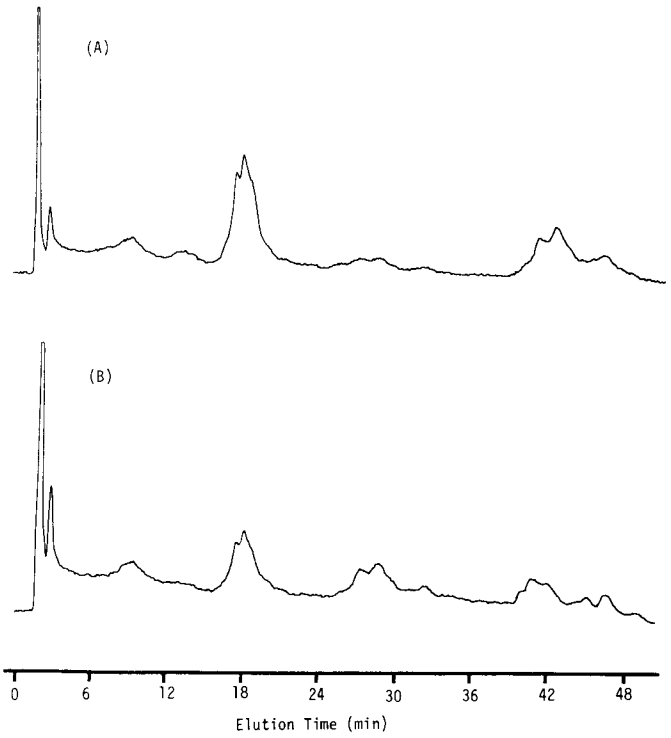
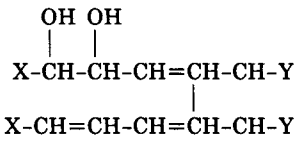
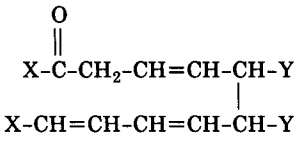
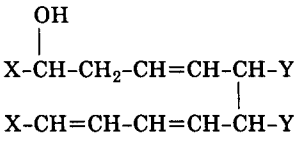
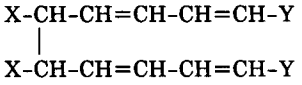
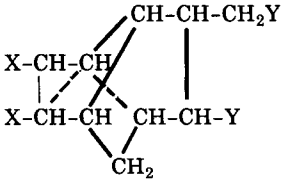
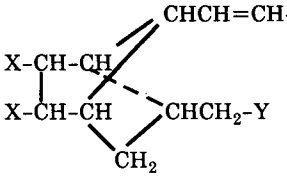
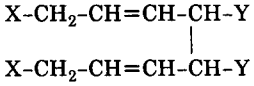
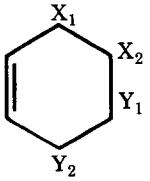


FIG. 6. HPLC chromatograms of dimers of oxidized oils A and B. Column, LC-18 (15 cm × 4.6 cm i.d., dp = 2 μm; mobile phase, acetonitrile at 1.0 ml/min, detection = UV at 205 nm.

retention times for dihydroxy and diketo dimers (RRT range =2.64-3.70). The mass spectral data obtained for this peak gave evidence for the presence of two different types of dimers in fraction I; the dihydroxy and ketodimer of the dehydrodimer of methyl linoleate as presented in Table 1. The molecular ion was not present either in the EI or CI spectra for the dihydroxydimer from the dehydrodimer of methyl linoleate. However, in the EI spectrum (Fig. 7) the following data indicated the presence of this dimer in fraction I. First, an ion at m/e 602 can result

TABLE 1

Dimer Structures Isolated from the Dimeric Fraction of Oxidized Soybean Oil Sample[a]

Fraction #	MW	# Isomers	Structure	Assignment
I, Peak #1	620	4		Dehydroxydimer of Me-LN[b]
Peak #2	602	8		Ketodehydrodimer of Me-LN[b]
II, Peak #1	604	8		Monohydrodimer of Me-LN[b]
Peak #2	586	6		Dehydrodimer of Me-LN[b]
III	588	6		Tricyclic dimers of Me-LN[b]
IV	586	6		Bicyclic dimer of Me-LN[b]
V, Peak #1	590	10		Dehydrodimer of Me-OL[b]
Peak #2	588	4		Thermal dimer of Me-LN[c]

[a] HPLC dimeric fractions are presented in Figure 4.

[b] For all dimers except the thermal dimer of Me-LN: X = $-(CH_2)_4CH_3$ and Y: $-(CH_2)_7COOCH_3$.

[c] Thermal dimer of Me-LN: X_1: $-(CH_2)_{4,5}CH_3$, X_2: $-CH_2)_{7,8}CH_3$, Y_1: $-(CH_2)_7COOCH_3$ or $-CH_2CH{=}CH(CH_2)_7COOCH_3$ and Y_2: $-(CH_2)_4CH_3$or $-CH_2CH{=}CH(CH_2)_7COOCH_3$.

from the molecular ion by H_2O loss. Second, the ions at m/e 503 and 521 can result from the molecular ion by subtraction of one or two hydroxyl groups and the alkyl fragment $-CH(CH_2)_4CH_3$. Third, the presence of an ion at m/e 293 would be indicative of the structure of the dehydrodimer of methyl linoleate. This ion results from cleavage of the C-C bond joining the two linoleate segments and can represent either the non-substituted moiety or the substituted segment with loss of the two hydroxyl groups. Similarly, the ions at m/e 309 or 311 can be explained as ions resulting from the cleavage of the C-C bond of the dimer with loss of a hydroxyl group from the resulting segment. The corresponding CI spectrum gave the characteristics fragments for M = 620. A very intense ion was observed at m/e 603 which results from loss of a hydroxyl group from the molecular ion. A weaker ion at m/e 586 resulting from loss of both hydroxyl groups from the molecular ion was also present.

In order to provide further evidence for the structure of the dihydroxy-dimer of the dehydrodimer of methyl linoleate, the trimethylsiloxy (TMS) derivative of fraction I (Fig. 4) was prepared and analyzed by GC-MS. In the EI spectrum (Fig. 8) the presence of ion at m/e 173, 201, 245, and 259 would indicate TMS substituted fragments that resulted from cleavage of the substituted C-C bond. The ion at m/e 381 represented the M/2 ion plus hydrogen resulting from the cleavage of the C-C bond joining the two linoleate segments of the dimer. The CI spectrum

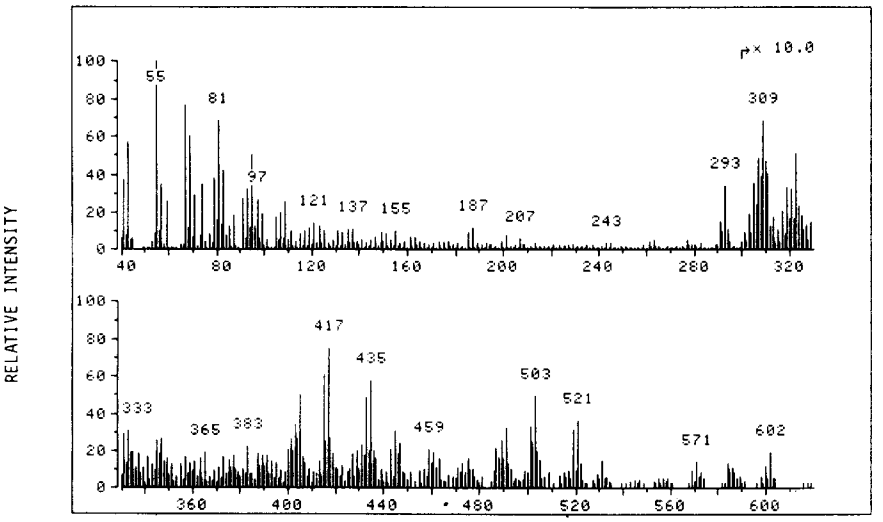


FIG. 7. EI mass spectrum of fraction I (from Figure 4).

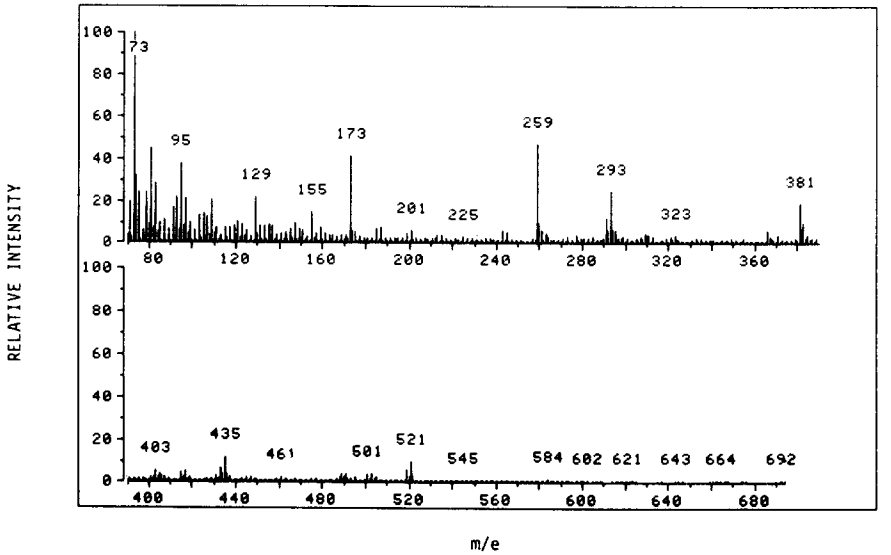


FIG. 8. EI mass spectrum of fraction I as TMS derivative (from Figure 4).

indicated ions at m/e 675 and 586 that would result from the loss from the molecular ion (M = 764) of one or two TMSO groups, respectively.

The presence of the ketodimer of the dehydrodimer of methyl linoleate was also suggested from the mass spectrum data of fraction I. A peak at m/e 602 in the EI spectrum may represent the molecular ion peak, whereas the base peak ion in the CI spectrum at m/e 603 may be the M + 1 peak for this dimer. The presence of peaks at M + 29 and M + 41 at m/e 631 and 643 presents additional tentative evidence for this dimeric structure (Fig. 8).

GC of dimer fraction II (Fig. 4) gave two peaks with RRT of 2.50 and 2.69. The EI and CI spectra of the first peak are presented in Figures 9 and 10. Analysis of the GC-MS data indicated the presence of the monohydroxy-dehydrodimer of methyl linoleate. A very small ion corresponding to the molecular ion at m/e = 604 was present in the EI spectrum. All other ions can be explained as originating from the dehydrodimer of methyl linoleate with additional loss of a hydroxyl group. The CI spectrum gave the characteristic ions for a dimer of molecular weight m/e = 604 (605 in CI mode). An intense ion at m/e 587 would result from the molecular ion by subtraction of the hydroxyl group.

The mass spectrum obtained from the second peak from the dimer fraction II (Fig. 4) is presented in Table 2. The EI spectrum showed a low intensity peak of m/e = 586. It also showed a base peak at M/2 = 293. Four series of peaks corresponding to the loss of alkyl fragments, alkyl fragments plus CH_3OH, alkyl fragments plus $2CH_3OH$,

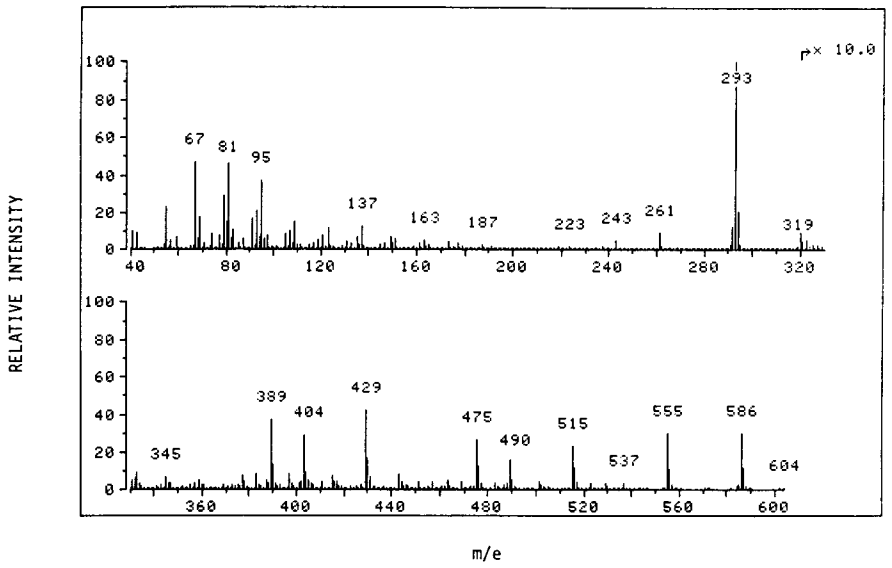


FIG. 9. EI mass spectrum of fraction II/peak 1 (from Figure 4).

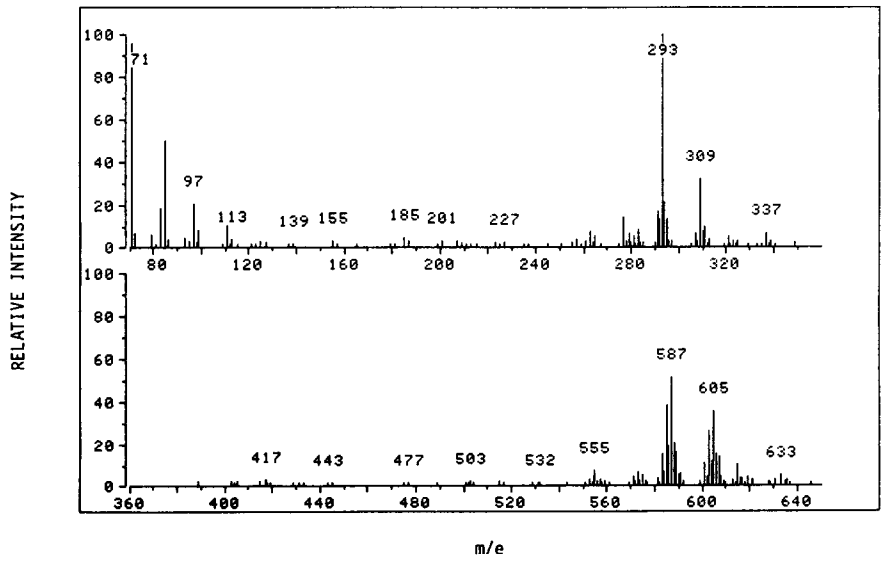


FIG. 10. CI mass spectrum of fraction II/peak 1 (from Figure 4).

and ester fragments were observed. The spectrum below m/e 260 showed the strong m/e 74 ion common to non-substituted methyl esters and the expected series of alkane and/or carboxymethoxyalkane peaks. The CI spectrum gave the characteristic ions at M + 1, M + 29, and M + 41 as expected for a dimer with molecular weight of 586.

Hydrogenation and GC-MS analysis of this fraction showed a molecular ion at m/e = 594, as was expected for a saturated dimer with one carbon to carbon linkage joining the two fragments. Ions at M-CH_3O, M-CH_3OH, and M-2CH_3O are seen (as expected) for the methyl ester of a dibasic acid. Cleavage at the carbon to carbon linkage was very pronounced at m/e 297. The intense ions at m/e 298 as well as the less intense at m/e 299 and 295 may be due to hydrogen rearrangements and isotope effects. Again, five series of peaks corresponding to ions represented the loss of alkyl groups, alkyl groups plus CH_3OH, alkyl groups plus 2CH_3OH, ester fragments, and esters fragments plus CH_3OH were present. The CI spectrum provided additional evidence for the molecular weight by giving the characteristic fragments for the hydrogenated dehydrodimer of methyl linoleate as described by Wheeler and White (21).

GC analysis of fraction III (Fig. 4) gave one peak at RRT of 2.60. The molecular ion was assigned at m/e 588 (Fig. 11). The CI spectrum verified the assignment of the molecular ion by giving the characteristic ions for a dimer of molecular weight of 588. Considering the retention characteristics as well as the mass spectral data of this dimer, two different types of dimers could represent this peak. A non-cyclic dimer with three double bonds or a saturated tricyclic dimer as described by Rushman and Simpson (22) and Sen Gupta and Scharmann (23). GC-MS analysis of the hydrogenated fraction gave a spectrum identical to that of the unhydrogenated dimer. This indicated the presence of the tricyclic dimer (Table 1). If the non-cyclic dehydrodimer of methyl linoleate with three double bonds was the dimer eluted in this fraction, then the spectrum of the hydrogenated material would

TABLE 2

Mass Spectral Data from Fraction II (Peak #2) Before and After Hydrogenation[a]

Fragment ions m/e (relative abundance)						Assignment
Electron ionization Fr. II (Pk #2)[b]						
586	(2.6)					M
555	(2.7)					M-OCH_3
293	(100.0)					M/2
261	(9.9)					M/2-CH_3OH
515	(6.,3)	483	(1.1)	451	(1.2)	M-X_1,M-(X_1+CH_3OH) M-(X_1+2CH_3OH)
502	(1.0)					M-X_2
489	(1.2)	457	(0.5)			M-X_3,M-(X_3+CH_3OH)
476	(1.4)	444	(1.4)			M-X_4,M-(X_4+CH_3OH)
475	(2.7)					M-X_5
429	(12.5)	389	(6.1)			M-Y_1,M-(Y_1+CH_3OH)
416	(1.0)					M-Y_2
403	(1.5)					M-Y_3
390	(2.3)					M-Y_4
Electron ionization Hydrogenated fr. II (Pk #2)[c]						
594	(5.5)					M
563	(6.9)	562	(9.4)	532	(1.3)	M-OCH_3,M-CH_3OH,M-2(OCH_3)
297	(82.1)					M/2
264	(27.0)	265	(29.0)			M/2-CH_3OH,M/2-OCH_3
523	(1.4)	491	(4.7)	459	(2.7)	M-X_1,M-(X_1+CH_3OH),M-(X_1+2CH_3OH)
		462	(1.1)	431	(1.5)	M-(X_2+CH_3OH),M-(X_2+2CH_3OH)
467	(1.1)	535	(3.3)	403	(1.7)	M-X_3,M-(X_3+CH_3OH),M-(X_3+2CH_3OH)
437	(2.2)	405	(2.0)			M-Y_1,M-(Y_1+CH_3OH)
409	(1.0)					M-Y_2
381	(4.3)	349	(2.1)			M-Y_3,M-(Y_3+CH_3OH)

[a] Fraction II as presented in Figure 4. GC-MS conditions as described in experimental section.

[b] Alkyl and ester fragments as following: X_1: -$(CH_2)_4CH_3$, X_2: -CH-$(CH_2)_4CH_3$, X_3: -CH=CH-$(CH_2)_4CH_3$, X_4: -CH-CH=CH-$(CH_2)_4CH_3$, X_5: -$(CH_2)_7CH_3$; Y_1: -$(CH_2)_7COOCH_3$, Y_2: -CH$(CH_2)_7COOCH_3$, Y_3: -CH=CH-$(CH_2)_7COOCH_3$, Y_4: -CH-CH=CH-$(CH_2)_7COOCH_3$.

[c] Alkyl and ester fragments as following: X_1: -$(CH_2)_4CH_3$, X_2: -$(CH_2)_6CH_3$ and X_3: $(CH_2)_9CH_3$; Y_1: -$(CH_2)_7COOCH_3$, Y_2: -$(CH_2)_9COOCH_3$ and Y_3: -$(CH_2)_{11}COOCH_3$.

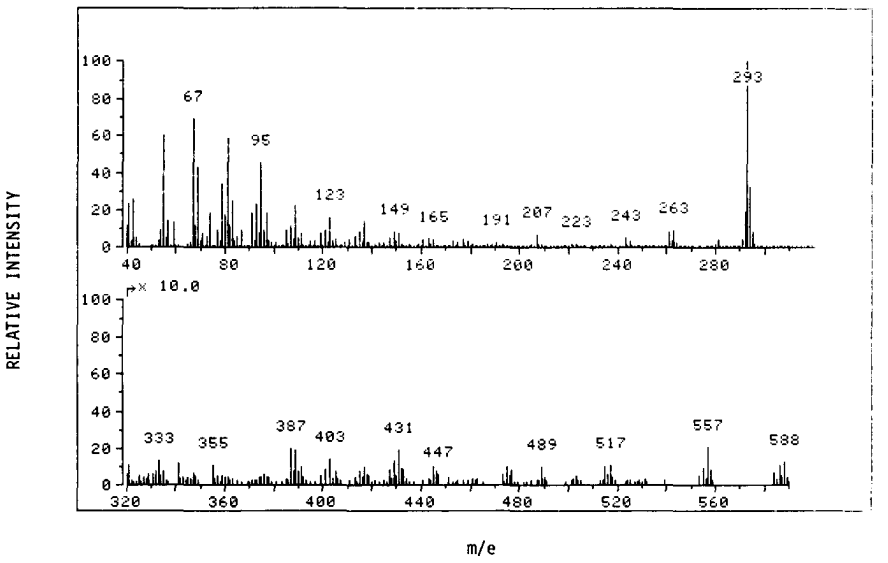


FIG. 11. EI mass spectrum of fraction III (from Figure 4).

have shown a molecular peak at m/e 594, which was not observed.

GC analysis of fraction IV (Fig. 4) gave one peak with a RRT of 2.71. The mass spectral data are indicated in Table 3. The EI mass spectrum showed a molecular ion at m/e = 586. Peaks corresponding to ions from the loss of alkyl, alkyl plus CH_3OH, ester, and ester plus CH_3OH were observed. The CI spectrum gave the fragments expected for a dimer of molecular weight of 586.

The spectrum of the hydrogenated fraction gave a molecular ion at m/e = 590. The parent ion at 590 is expected for the saturated bicyclic dimer of methyl linoleate. The CI spectrum gave the characteristic molecular ion for a dimer of molecular weight of 590. Major peaks resulting from the loss of either alkyl or ester fragments from the molecular ion at 590 were also observed. All these data are in accordance with the structure of the bicyclic dimer of methyl linoleate as described by Wheeler and White in their synthetic work (21).

Analysis of fraction V (Fig. 4) by gas chromatography gave two unresolved peaks of RRT 2.23 and 2.63. The GC-MS analysis data of the first peak is presented in Table 4 and they are similar to those obtained for the synthetic dehydrodimer of methyl oleate described by Paschke *et al.* (24). In the EI spectrum the parent ion at m/e 590 was not observed, probably because there are many C=C bonds where cleavage could occur. The ion at m/e 559 due to the loss of OCH_3 radical is observed. Very intense ions were seen at 295 (base peak, M/2) and 294 (hydrogen rearrangement). The cleavage of the bond joining the two oleate fragments is very pronounced since this linkage is between two tertiary carbons and is allylic to two double bonds. Loss of the C_7H_5 and C_8H_1 alkane fragments as well as $C_6H_{12}COOCH_3$ and $C_7H_{14}COOCH_3$ is prominent since these groups are attached to tertiary carbons and are allylic to the double bonds. The molecular weight of the dehydrodimer was verified by the characteristic ions in the CI spectrum.

TABLE 3

Mass Spectral Data from Fraction IV Before and After Hydrogenation[a]

Fragment ions m/e (rel. abundance)	Assignment
Electron ionization—Fr. IV[b]	
586(28.6)	M
555(11.3)	M-OCH_3
293(22.7)	M/2
262 (2.1)	M/2-OCH_3
515(42.0)	M-X_1
483 (7.6)	M-[+CH_3OH] X_1
451 (6.4)	M-[+2CH_3OH] X_1
443(14.0)	M-Y_1
529(77.2)	M-Y_2
389(25.0)	M-[+CH_3OH] Y_3
Electron ionization Hydrogenated IV[c]	
590 (3.5)	M
558 (6.9) 559 (6.0)	M-CH_3O, M-CH_3OH
519 (3.5) 487(10.7)	M-X_1, M-[alkyl+CH_3OH]
473 (4.8)	M-X_2
447 (3.1)	M-Y_1
433 (7.6)	M-Y_2
419 (4.0)	M-Y_3
405 (2.1)	M-Y_4

[a] Fraction IV as presented in Figure 4. GC-MS conditions as described in experimental section.

[b] Alkyl and ester fragments as following: X_1: -$(CH_2)_4CH_3$; Y_1: -$(CH_2)_6COOCH_3$, Y_2: -$(CH_2)_7COOCH_3$, Y_3: -$(CH_2)_8COOCH_3$.

[c] Alkyl and ester fragments as following: X_1: -$(CH_2)_4CH_3$, X_2: -$(CH_2)_5CH_3$; Y_1: -$(CH_2)_6COOCH_3$, Y_2: -$(CH_2)_7COOCH_3$, Y_3: -$(CH_2)_8COOCH_3$, Y_4: -$(CH_2)_9COOCH_3$.

TABLE 4

Mass Spectral Data from Fraction V (Peak #1) Before and After Hydrogenation[a]

Fragment ions m/e (rel. abundance)	Assignment
Electron ionization—Fr. V (Pk #1)[b]	
559 (4.3)	$M-OCH_3$
295 (35.6) 294(100.0)	M/2, M/2-1
264 (8.1) 263 (30.2)	$M/2-OCH_3$, $M/2-CH_3OH$
471 (0.8)	$M-X_1$
477 (1.0)	$M-X_2$
447 (1.2)	$M-Y_1$
433 (1.9)	$M-Y_2$
Electron ionization—Hydrogenated Fr. V (Pk #1)[c]	
594 (2.6)	M
562 (3.8) 563 (3.5) 532 (1.3)	$M-CH_3OH$,$M-OCH_3$,$M-2(OCH_3)$
297(100.0) 298 (92.2) 299(57.8)	M/2,M/2+1,M/2+2
265 (40.6) 265 (39.6)	$M/2-CH_3OH$,$M/2-(CH_3OH+H)$
463 (2.7) 431 (2.0)	$M-(X_1+CH_3OH)$,$M-(X_1+2CH_3OH)$
449 (2.7) 417 (2.0)	$M-(X_2+CH_3OH)$,$M-(X_2+2CH_3OH)$
435 (2.9) 403 (2.4)	$M-(X_3+CH_3OH)$,$M-(X_3+2CH_3OH)$
421 (2.9) 389 (1.6)	$M-(X_4+CH_3OH)$,$M-(X_4+2CH_3OH)$
451 (2.0) 419 (1.6)	$M-Y_1$,$M-(Y_1+CH_3OH)$
437 (2.4) 405 (1.2)	$M-Y_2$,$M-(Y_2+CH_3OH)$
423 (2.7) 391 (1.2)	$M-Y_3$,$M-(Y_3+CH_3OH)$
409 (2.7) 377 (1.2)	$M-Y_4$,$M-(Y_4+CH_3OH)$

[a] Fraction V as presented in Figure 4. GC-MS conditions as described in experimental section.

[b] Alkyl acid ester fragments as following: X_1: $-(CH_2)_6CH_3$, X_2: $-(CH_2)_7CH_3$; Y_1: $-(CH_2)_6COOCH_3$, Y_2: $-(CH_2)_7COOCH_3$.

[c] Alkyl and ester fragments as following: X_1: $-(CH_2)CH_3$, X_2: $-(CH_2)_7CH_3$, X_3: $-(CH_2)_8CH_3$ and X_4: $-(CH_2)_9CH_3$; Y_1: $-(CH_2)_6COOCH_3$, Y_2: $-(CH_2)_7COOCH_3$, Y_3: $-(CH_2)_8COOCH_3$, Y_4: $-(CH_2)_9COOCH_3$.

TABLE 5

Mass Spectral Data from the Fraction V (Peak #2) Before and After Hydrogenation[a]

Fragment ions m/e (rel. abundance)	Assignment	Fragment ions m/e (rel. abundance)	Assignment
Electron ionization Fr. V (Pk #2)[b]		Electron ionization—Hydrogenated Fr. V (Pk #2)[c]	
588 (12.2)	M	592 (8.4)	M
557 (13.9)	$M-OCH_3$	561 (17.8)	$M-OCH_3$
294(100.0)	M/2	560 (34.8)	$M-CH_3OH$
263 (7.3)	$M/2-OCH_3$, $M/2-CH_3OH$	521 (5.4)	$M-X_1$
517 (21.3)	$M-X_1$	489 (42.4)	$M-[+CH_3OH]\ X_1$
503 (14.3)	$M-X_2$	507 (3.2)	$M-X_2$
477 (11.5)	$M-X_3$	475 (27.3)	$M-[+CH_3OH]\ X_2$
491 (5.9)	$M-X_4$	493 (4.2)	$M-X_3$
431 (37.3)	$M-Y_1$	461 (30.8)	$M-[+CH_3OH]\ X_3$
445 (17.1)	$M-Y_2$	479 (5.2)	$M-X_4$
417 (27.5)	$M-Y_3$	447 (55.1)	$M-[+CH_3OH]\ X_4$
391 (26.5)	$M-Y_4$	435 (16.7)	$M-Y_1$
321 (27.5)	$M-[X_1 + Y_5-H]$	421 (12.3)	$M-Y_2$
		407 (12.9)	$M-Y_3$
		393 (19.3)	$M-Y_4$
		321 (60.8)	$M-[X_4+Y_5+H]$ or $M-[X_4+Y_1+H]$

[a] Fraction V as presented in Figure 4. GC-MS conditions as described in experimental section.

[b] Alkyl and ester fragments as following: X_1: $-(CH_2)_4CH_3$, X_2: $-(CH_2)_5CH_3$, X_3: $-CH_2CH=CH(CH_2)_4CH_3$, X_4: $-CH=CH(CH_2)_4CH_3$; Y_1: $-(CH_2)_7COOCH_3$, Y_2: $-(CH_2)_6COOCH_3$, Y_3: $-(CH_2)_8COOCH_3$, Y_4: $-(CH_2)_7CH=CH(CH_2)_7COOCH_3$, Y_5: $-CH=CH(CH_2)_7COOCH_3$.

[c] Alkyl and ester fragments as following: X_1: $-(CH_2)_4CH_3$, X_2: $-(CH_2)_5CH_3$, X_3: $(CH_2)_6CH_3$, X_4: $-CH=CH(CH_2)_4CH_3$; Y_1: $-(CH_2)_7COOCH_3$, Y_2: $-(CH_2)_8COOCH_3$, Y_3: $-(CH_2)_9COOCH_3$, Y_4: $-(CH_2)_{10}COOCH_3$, Y_5: $-CH=CH-(CH_2)_7COOCH_3$.

The hydrogenated dimer provided further evidence for the establishment of the dehydrodimer structure. The parent ion was observed at m/e = 594, as expected for the saturated dimer, with one carbon to carbon linkage joining the two C-18 segments. Ions at $M-CH_3O$, $M-CH_3OH$, and $M-2CH_3O$ are seen and could be expected for the methyl ester of a dibasic acid. Cleavage at the bond joining the two chains is very pronounced, with the ion M/2 297 being the base peak in the spectrum. Ions at m/e 296, 298, and 299 must be due to hydrogen rearrangements as well as isotope effects. Five series of ions corresponding to the loss of alkyl fragments, alkyl plus CH_3OH, alkyl plus $2CH_3OH$, ester, and ester plus CH_3OH. The CI spectrum showed the characteristic ions for a dimer of molecular weight 594.

The EI spectrum of the second peak of dimer fraction V (Fig. 4) showed the parent peak at m/e 588 (Table 5). A mass of 588 would result from either Diels-Alder isomerization reactions or free radical coupling of radicals of molecular weights 293 and 295.

The following data indicated that this dimer contains a Diels-Alder structure as described by Wheeler and White (21). A base peak at M/2 = 294 due to a reverse or retro Diels-Alder reaction as has been observed with other analogous Diels-Alder adducts. On hydrogenation, this dimer did not give an M/2 peak since it was no longer a Diels-Alder adduct structure. A strong peak at m/e = 321 corresponding to the simultaneous loss of the two groups in the 2,3 positions of the Diels-Alder dimeric structures with hydrogen transfer. Relatively strong peaks corresponding to the loss of either alkyl or ester groups on the cyclohexane rings. In the chemical ionization spectrum, the expected M + 1, M + 29, and M + 41 ions were observed.

In the EI spectrum of the hydrogenated dimer the peak at m/e 592 corresponds to the hydrogenated monocyclic dimer expected from the Diels-Alder dimerization reaction. This is indicated by the absence of an intense ion at M/2 = 296, the relatively intense ion at m/e 321 corresponding to loss of the two groups in the 2,3 positions of the Diels-Alder adduct with hydrogen transfer, the relatively strong ions due to loss of expected alkyl groups as well as alkyl groups plus methanol, and the relatively intense ions corresponding to the loss of ester containing side chains.

Loss of C_5H_{11}, C_6H_{13}, and C_8H_{17} alkyl groups as well as $(CH_2)_7COOCH_3$, $(CH_7)_8COOCH_3$, and $(CH_2)_{10}COOCH_3$ indicates that either the 9 or 12 double bond of the unconjugated linoleate acted as dienophile. Loss of C_7H_{15} and $(CH_2)_9COOCH_3$ indicated that the C_{10} double bond of 10, 12, and the C_{11} double bond of 9,11 conjugated linoleate isomers acted as dienophiles.

The mass spectra of the dimeric fractions collected from sample B (Fig. 5) were identical to those obtained from the dimeric fraction of sample A (Fig. 5) except that fraction II gave only one component which was identified as that of the dehydrodimer of methyl linoleate. No evidence was obtained for the presence of the monohydroxy of the dehydrodimer of methyl linoleate as in sample A.

The chemical similarity of the composition of dimeric fractions from used oil samples A and B indicate a major role of the fatty acid composition of the oil on the formation of the dimeric compounds under thermal-oxidative reactions. The results of this study suggest that for the same frying oil medium, variation in the oxidation conditions do not appear to result in qualitative differences in the types of the dimers formed in the oxidized oil. Variable fatty acid composition frying fats and oils, depending upon their linoleic and linolenic acid content would be expected to result in quantitative differences in the distribution of dimeric fatty acid structural moieties formed during heating and exposure to air.

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