Chemical Reactions Involved in the Deep-Fat Frying of Foods: VIII. Characterization of Nonvolatile Decomposition Products of Triolein[1]

M.M. PAULOSE[2] and **STEPHEN S. CHANG**, Department of Food Science,
Cook College, Rutgers, The State University of New Jersey,
New Brunswick, New Jersey 08903

ABSTRACT

Triolein was heated under simulated deep-fat frying conditions at 185 C for 74 hr. The thermally oxidized triolein was converted into methyl esters and then fractionated by urea exclusion. The urea adduct-forming ester (89.2%) was found to be methyl oleate unchanged by the frying treatment. The non-urea adduct-forming esters (10.8%) were further fractionated by silicic acid column chromatography into nine fractions with molecular weights ranging from 304 to 742. Physical and chemical analyses of the fractions indicated that some of them contained oxygen atoms which could not be accounted for by ordinary functional group analyses. The polymers isolated were formed by both carbon-to-carbon and carbon-to-oxygen linkages. The nonpolar dimers were further purified by thin layer and gas chromatography. Structure elucidation revealed that they consisted of a noncyclic dimer and a noncyclic dimer containing a carbonyl group, each of which amounted to 1.36% of the triolein originally used. The polar polymers were studied by depolymerization and the analysis of the depolymerized products. It was estimated that the triolein used for simulated deep fat frying contained 1.1% trimers formed through carbon-to-carbon linkages, 1.9% dimers and trimers joined through carbon-to-carbon linkages, and 3.1% dimers and trimers joined through carbon-to-oxygen or carbon-to-carbon linkages in the same molecule and also dimers and trimers in which all the monomeric units were joined through carbon-to-oxygen linkages. The precise form of the oxygen linkages are not known. However, the fact that it is not cleavaged by HCl and HI suggests ether linkages.

INTRODUCTION

Nonvolatile decomposition products (NVDP) of triglycerides produced during deep-fat frying have been reported in numerous publications to have an adverse effect upon human health (1). They also play an important practical role in affecting the flavor, flavor stability, color, and texture of deep-faty fried foods. The understanding of the chemical structures of these compounds are also essential for the development of simple and effective analytical methods for the quality control of the fats and oils used for deep-faty frying in households, restaurants, institutions, and food manufacturing plants.

The triglycerides of frying fats and oils are usually composed of saturated, mono-unsaturated, and di-unsaturated fatty acids. The characterization of the NVDP produced by trilinolein has been reported in a previous publication by Paulose and Change (2). The present publication reports the characterization of such compounds produced by triolein. A subsequent paper will report those formed through tristearin.

EXPERIMENTAL PROCEDURES

Material Used

The triolein used in this investigation was made from ethyl oleate which was obtained from ethyl esters of olive oil. The triglycerides were prepared by transesterification with glycerol using an alkaline catalyst and purified by crystallization from acetone and finally freed from solvent by vacuun steam deodorization up to 180 C. Thin layer chromatographic analysis indicated that the triolein contained 0.3% of ethyl oleate and 0.5% of diolein. Gas chromatography of the total methyl esters of the triolein showed that they were 99.9% oleate.

Simulated Deep-Fat Frying

The triolein (2128 g) was treated under simulated deep-fat frying conditions at 185 C for 74 hr using the same apparatus and procedure as reported previously for trilinolein (2). In this method, steam was periodically injected into the heated triolein to simulate frying.

Isolation and Preliminary Fractionation of NVDP

The triolein, after treatment under simulated frying conditions for 74 hr, was freed from VDP by vacuum steam distillation at 150 C under 0.01 mm Hg for 1 hr. Free fatty acids left in the products were removed by treatment with Amberlite IRA-400 ion exchange resin according to the method of Hornstein et al. (3). The treated tiolein was then converted into methyl esters by transesterification using sodium methoxide as a catalyst (4). The methyl esters were then separated into urea-adduct-forming (AF) esters and mon-urea-adduct-forming (NAF) esters by the method of Firestone et al. (5).

The NAF esters were fractionated by liquid chromatography with the use of silicic acid according to the method of Sahasrubudhe and Chapman (6). By eluting with hexane containing stepwise increasing amounts of ethyl ether, as shown in Figure 1, the NAF esters were separated into eight fractions, 1-8. Each of the first seven fractions was rechromatographed on a silicic acid column, and the major peak material was collected as fraction 1B-7B. Fraction 8 was rechromatographed on a silicic acid column to obtain fractions 8A and 8B, with the use of ethyl ether and methanol as eluents, respectively.

Isolation of Dimers

Fraction 1B from silicic acid column chromatography was further separated by preparative TLC using a 1 mm thick 20 x 20 cm Silica Gel G plate. Approximately 75 mg of fraction 1B was applied to each plate, and elution was done with benzene according to the method of Paschke et al. (7). A portion of the plate was sprayed with 2,7-dichlorofluoresceine and was viewed under UV light. Three

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[1]Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey.

[2]Present address: Regional Research Laboratory, Hyderabad 9, India.

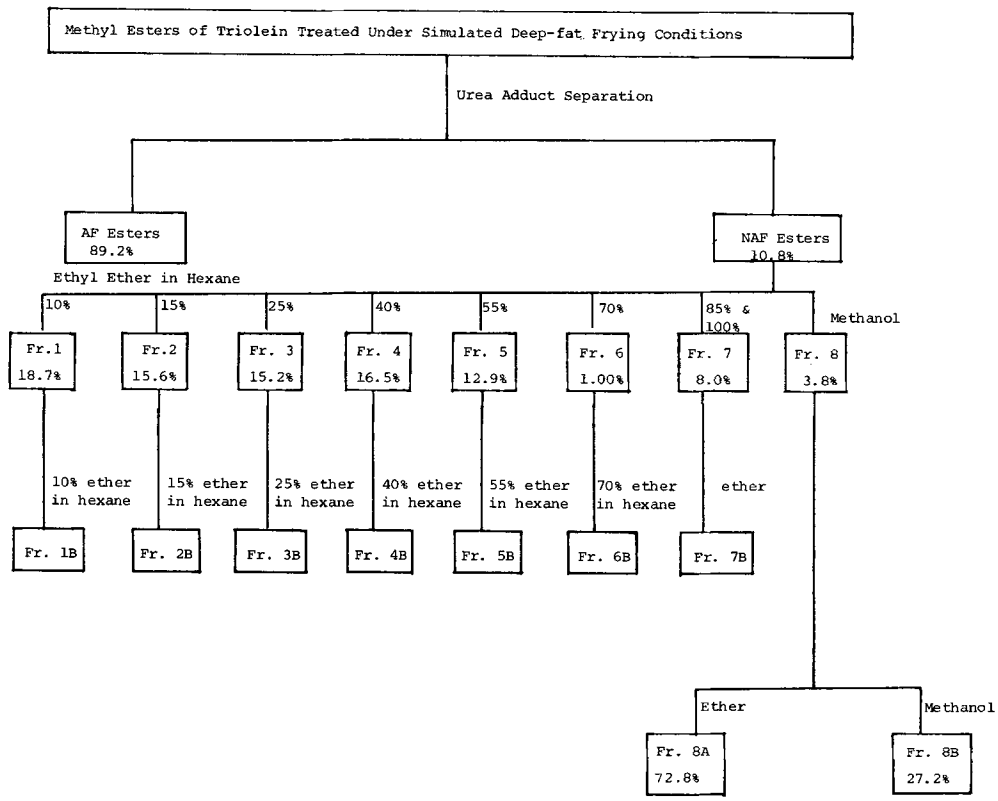

FIG. 1. Silicic acid column chromatography of the non-urea-adduct-forming esters of triolein treated under simulated deep-fat frying conditions.

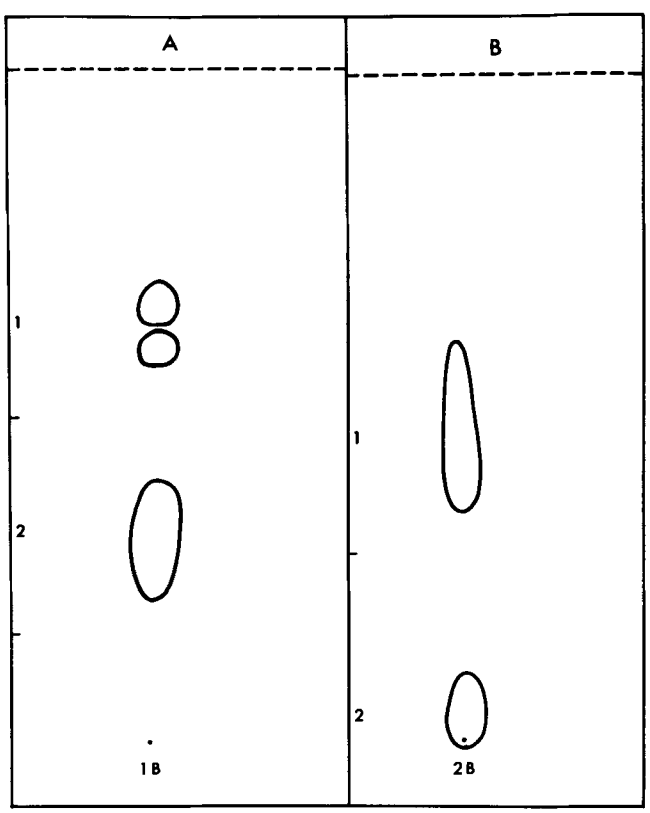

FIG. 2. Thin layer chromatograph of fractions 1B and 2B on Silica Gel G.

well separated spots were obtained as shown in Figure 2. Each of the three spots was scraped from the unsprayed portion of the plate. The sample was then recovered by extracting with ethyl ether. Preparative TLC was repeated a number of times until a sufficient amount of each fraction was accumulated. In order to improve its purity, fraction 1B2 was rechromatographed twice with a thin layer Silica Gel G plate.

Fraction 2B, from silicic acid column chromatography, was further separated into two fractions – 2B1 and 2B2 in similar manner.

Depolymerization Studies

Two different depolymerization procedures were used. The method of Chang and Kummerow (8) using 2.5 N dry hydrochloric acid in anhydrous methanol was used to break polymers joined through peroxide linkages. The method of Williamson (9) using anhydrous hydroiodic acid was used to break polymers joined through ether linkages.

Fractions 3B-7B were each depolymerized by the two procedures. The depolymerized product obtained from each of the four fractions by treating with hydroiodic acid was dehalogenated with zince dust in acetic acid (9) and then esterified with anhydrous methanol using sulfuric acid as a catalyst. The methyl esters of the depolymerized product of each fraction were then separated into four fractions by TLC using Silica Gel G plates with benzene as the developer (Fig. 3).

Analytical Methods

Iodine value, free fatty acids, peroxide value, oxirane oxygen, and photometric color were determined according to AOCS official methods (10). Saponification value, hydroxyl value, and conjugated diene were determined according to the method described by Mehlenbacher (11). Carbonyl value was analyzed by the method of Bhalerao et al. (12).

Viscosity was measured with a Cannon Fenske Viscometer (No. 200, Cannon Instrument Co.). Molecular weight was determined with a Mechrolab Vapor Pressure Osmom-

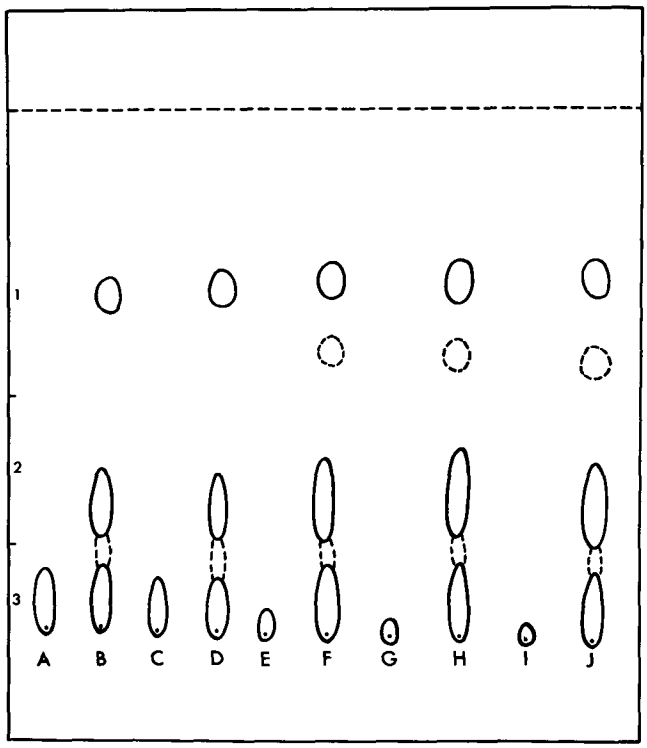

FIG. 3. Thin layer chromatographic fractionation of the depolymerized products of the polar polymers. A, C, E, G, and I are fractions 3B1, 4B1, 5B, 6B, and 7B, respectively. B, D, H, and J are their respective depolymerized product.

TABLE I

Physical and Chemical Changes of Triolein during Simulated Deep-Fat Frying

	Triolein	Heated triolein
Viscosity (centistokes, 30 C)	56.2	101.8
Refractive index (40 C)	1.4632	4.4655
Color (AOCS, photometric)	5.8	62.5
Free fatty acids (%)	Nil	3.9
Iodine value (WIJS)	85.0	78.1
Peroxide value (meq/kg)	0.9	3.4
NAF esters	---	10.8

eter, Model 301A. Elemental analysis was carried out by Schwarzkopf Micro Analytical Lab., Woodside, NY.

Dehydration analysis was carried out by heating the samples, fractions 1B-7B, at 280 C for 5 hr under a nitrogen atmosphere with 10% Pd on charcoal as a catalyst. Absorption of the dehydrogenated product in the UV region was measured with a Beckman DBG spectrophotometer.

IR studies were carried out with a Beckman IR-8 IR spectrophotometer. NMR studies were conducted with a Varian Associates A-60 instrument.

RESULTS AND DISCUSSION

Deterioration of Triolein during Simulated Deep-Fat Frying

After the triolein was used for simulated deep-fat frying at 185 C for 74 hr, ther were significant increases in viscosity, refractive index, color, and free fatty acids (Table I). The peroxide value only increased to 3.4 meq/kg. This was expected because peroxides tend to decompose at the frying temperature. The NAF esters were increased to 10.8%. Under identical conditions, trilinolein formed 26.3% of NAF esters (2).

Gas chromatographic analysis of the urea-adduct-forming (AF) esters with a 20 ft x 1/8 in OD stainless-steel column packed with 23% DEGS on 45/60 mesh Chromosorb W yielded a gas chromatogram which was identical to that of the methyl esters obtained from the triolein prior to the simulated deep-fat frying. Although chain cleavage as well as polymerization may take place during the conditions used for thermal oxidation, 89.2% of the fatty acids were not attacked after 74 hr of treatment under simulated deep-fat frying conditions.

Under identical conditions, only 73.7% of the fatty acids in trilinolein remained unchanged (2).

Characterization of Liquid Chromatographic Fractions

The nine fractions of NAF esters were not pure compounds. Nevertheless, they were analysed to give a general idea of the chemical nature of the NVDP produced during deep-fat frying (Table II). The AF esters were also analyzed for comparison purposes.

The molecular weights of these fractions ranged from 304 to 742 indicating the presence of trimers of methyl oleate with either some breakage of the fatty acid chain or contamination with dimers. Corresonding fractions obtained from trilinolein were shown to have slightly higher

TABLE II

Analysis of AF Esters and Fractions of NAF Esters Obtained from Triolein after Being Used for Simulated Deep-Fat Frying

	AF esters	Fractions of NAF esters								
		1B	2B	3B	4B	5B	6B	7B	8A	8B
Molecular weight	295	464	556	518	573	642	705	742	589	304
Carbon, %	—	75.08	74.96	72.57	72.76	73.05	73.11	69.66	72.95	53.92
Hydrogen, %	—	12.04	11.53	11.28	10.98	10.89	10.92	10.30	10.94	9.47
Oxygen, % (by difference)	—	12.88	13.51	16.15	16.26	16.06	15.97	20.04	16.11	36.61
Iodine value	85.8	64.7	78.3	74.7	67.8	56.8	56.1	55.7	58.7	24.7
Double bond/mole	1.00	1.18	1.72	1.52	1.53	1.44	1.56	1.63	1.36	0.03
Saponification value	202	196	187	195	201	218	222	225	209	109
Ester/mole	1.06	1.62	1.85	1.80	2.05	2.50	2.79	2.98	2.20	0.59
Hydroxyl value	Nil	Nil	13.45	16.8	55.7	60.0	90.4	131.6	115.0	388.0
Hydroxyl/mole	Nil	Nil	0.13	0.16	0.57	0.69	1.14	1.74	1.27	2.10
Carbonyl value	Nil	Nil	430	868	523	158	Nil	Nil	Nil	Nil
Carbonyl/mole	Nil	Nil	0.24	0.45	0.30	0.10	Nil	Nil	Nil	Nil
Peroxide value	9.0	10.2	36.4	41.5	56.0	59.0	68.0	85.0	30.5	21.0
Peroxide/mole	0.00	0.01	0.02	0.02	0.03	0.04	0.05	0.06	0.02	0.01
Conjugated diene, %	0.13	1.01	3.64	3.60	5.05	5.97	6.87	6.07	6.67	5.60
Oxyrane oxygen	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

TABLE III

Distribution of Oxygen among Functional Groups in Fractons of NAF Esters Obtained from Triolein after Being Used for Simulated Deep-Fat Frying

Functional groups	Fractions of NAF esters								
	1B	2B	3B	4B	5B	6B	7B	8A	8B
Hydroxyl	Nil	0.13	0.16	0.57	0.69	1.14	1.74	1.27	2.10
Ester	3.24	3.70	3.60	4.10	5.00	5.58	5.96	4.40	1.18
Carbonyl	Nil	0.24	0.45	0.30	0.10	Nil	Nil	Nil	Nil
Peroxide	0.01	0.04	0.04	0.06	0.08	0.10	0.12	0.04	0.01
Epoxy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Total oxygen atoms from functional group analysis	3.25	4.11	4.25	5.03	5.87	6.82	7.82	5.71	3.29
Oxygen atoms calculated by elemental analysis	3.74	4.69	5.23	5.82	6.44	7.04	9.29	5.93	6.96
Unaccounted oxygen atoms	0.49	0.58	0.98	0.79	0.57	0.22	1.47	0.22	3.67

TABLE IV

Analysis of Fractions 1B2B and 2B1B

	Fraction 1B2B	Fraction 2B1B
Molecular weight	581	589
Carbon, %	76.81	75.06
Hydrogen, %	11.95	11.56
Oxygen, % (by difference)	11.24	13.38
Iodine value	89.3	84.5
Double bond/mole	2.04	1.96
Saponification value	189.4	194.1
Ester/mole	1.96	2.04
Carbonyl value	Nil	1645
Carbonyl/mole	Nil	0.97

TABLE V

TLC Fractionation of the Depolymerized Products of the Polar Polymeric Fractions

		R_f	%
Fractionation of 3B1:			
	3B1A	0.64	9.4
	3B1B	0.24	63.4
	3B1C	0.06	27.2
Fractionation of 4B1:			
	4B1A	0.64	14.2
	4B1B	0.24	26.2
	4B1C	0.06	59.6
Fractionation of 5B:			
	5B1	0.64	15.8
	5B2	0.24	31.6
	5B3	0.06	52.6
Fractionation of 6B:			
	6B1	0.64	16.3
	6B2	0.24	29.0
	6B3	0.06	54.7
Fractionation of 7B:			
	7B1	0.64	20.5
	7B2	0.24	19.6
	7B3	0.06	59.9

molecular weights of 485-833 (2).

Most of the fracitons were highly polar containing as many as two hydroxyl groups per molecule. The number of oxygen atoms calculated from the functional group analysis cannot account for the number of oxygen atoms calculated from elemental analysis (Table III). Such oxygen atoms may be present in the molecule either as linkages joining the monomeric unit or in some functional groups which were not analyzed.

The infrared spectra of all the fractions showed typical absorption of fatty acid methyl esters. Fraction 1B showed a strong band at 10.35μ suggesting the presence of *trans* unsaturation. The hydroxy band at 2.9μ was weak in fractions 1B, 2B, and 3B, but quite strong in the rest of the fractions. In fraction 8B, this absorption was shifted to 2.95μ.

Analysis of cyclic structures indicated a distinct difference between triolein and trilinolein when each was treated under identical deep-fat frying conditions. The monoene fatty acid triglycerides formed no cyclic decomposition products while the diene fatty acid triglycerides formed 4.9% of cyclic dimers (2).

Purification and Identification of Dimer Fractions

Two dimer fractions, 1B and 2B, were further purified by thin layer and gas chromatography. Fraction 1B yielded three spots by thin layer chromatography. Two spots with an R_f value of 0.6 and 0.62 were collected together as Fraction 1B1, and one spot with an R_f value of 0.31 was collected as 1B2 (Fig. 2).

Fraction 1B1 constituted 27.4% of fraction 1B, or 0.55% of the total thermally oxidized triolein. Gas chromatography of this fraction with a 6 ft x ¼ in OD column of 15% DEGS on Anachrom ABS yielded one major peak (77.1%) and several minor peaks. The retention time and IR spectrum of this fraction were identical to those of methyl oleate.

Fraction 1B2 constituted 72.6% of fraction 1B. When it was gas chromatographed with 3 ft x ¼ in OD column of 1% OV-1 on 60/80 mesh Chromosorb G and temperature programmed from 150-300 C, one major fraction (1B2B) and five minor fractions (1B2A) were collected. The major fraction, 1B2B, constituted 92.5% of 1B2 and was equivalent to 1.36% of the total thermally oxidized triolein.

Analyses of 1B2B are shown in Table IV, which agrees well with a noncyclic dimer of methyl oleate joined by a carbon-to-carbon linkage. Paschke et al. (13) prepared a dehydro-dimer of methyl oleate and its structure was determined as a model of nonring dimer. They reported two double bonds per molecule. Gupta (14) suggested the presence of a dienoic dimer in thermally treated methyl oleate.

The IR spectrum of this fraction was similar to that of methyl oleate. A strong absorption band at 10.35μ indicated the presence of *trans* configuration. Its NMR spectrum showed the following proton signals: around 4.8τ (double bond), 6.4τ (methyl ester), around 7.8τ (methylene group adjacent to ester group), 8.65τ (methylene group), and around 9.0τ (terminal methyl group).

Fraction 2B yielded two spots; 2B1 with an R_f value of 0.47 and 2B2 with an R_f value of 0.05 when thin layer chromatographed. Fraction 2B1 constituted 88.8% of fraction 2B. When it was gas chromatographed in the same manner as fraction 1B2, it yielded one minor peak (2B1A) and a major peak (2B1B) which corresponds to 89.3% of fraction 2B1 which corresponds to 1.36% of the thermally oxidized triolein. In order to test whether there is any decomposition during the gas chromatography, the collected

TABLE VI

Analysis of the Dimer and Trimer Fractions Obtained by Depolymerization of the Polar Polymeric Fractions

	3B1B	3B1C	4B1B	4B1C	5B2	5B3	6B2	6B3	7B2	7B3
Molecular weight	441	606	506	662	473	647	544	796	512	768
Carbon %	74.42	73.65	74.47	72.25	72.11	70.96	75.57	72.30	75.70	72.63
Hydrogen, %	11.62	11.21	11.55	11.01	11.34	10.61	11.69	10.70	11.74	10.76
Oxygen, % (by difference)	13.96	15.14	13.98	16.74	16.55	18.43	12.74	17.00	12.56	16.61
Iodine value	44.2	33.0	42.4	34.8	32.7	30.1	40.8	37.3	46.3	47.1
Double bonds/mole	0.77	0.79	0.84	0.91	0.61	0.77	0.88	1.17	0.93	1.43
Saponification value	202	221	203	218	228	218	207	219	200	225
Ester/mole	1.55	2.39	1.83	2.58	1.93	2.52	1.87	3.10	1.83	3.08
Hydroxy value	Nil	Nil	Nil	Nil	Nil	28.1	10.6	100.1	16.2	58.3
Hydroxy/mole	Nil	Nil	Nil	Nil	Nil	0.32	0.10	0.90	0.15	0.50
Carbonyl value	1349	608	289	518	142	241	Nil	Nil	Nil	Nil
Carbonyl/mole	0.60	0.38	0.14	0.34	0.07	0.16	Nil	Nil	Nil	Nil
Peroxide value	181	190	707	748	1594	876	138	155	135	236
Peroxide/mole	0.08	0.11	0.36	0.50	0.75	0.57	0.08	0.12	0.07	0.18

TABLE VII

Distribution of Oxygen among Functional Groups of the Fractions Obtained by TLC Fractionation of the Depolymerized Samples

Functional groups	3B1B	3B1C	4B1B	4B1C	5B2	5B3	6B2	6B3	7B2	7B3
Ester	3.10	4.78	3.66	5.16	3.86	5.04	3.74	6.20	3.66	6.16
Hydroxyl	Nil	Nil	Nil	Nil	Nil	0.32	0.10	0.90	0.15	0.50
Carbonyl	0.60	0.38	0.14	0.34	0.07	0.16	Nil	Nil	Nil	Nil
Peroxide	0.16	0.22	0.72	1.00	1.50	1.14	0.16	0.24	0.14	0.36
Total number of atoms calculated from functional group analysis	3.86	5.38	4.52	6.50	5.43	6.66	4.00	7.34	3.95	7.02
Total number of oxygen atoms calculated from elemental analysis	3.85	5.73	4.41	6.93	4.90	7.45	4.33	8.45	4.02	7.97
Difference	+0.01	+0.35	-0.11	+0.43	-0.53	+0.79	+0.33	+1.11	+0.07	+0.95

2B1B was rechromatographed and only a single peak was observed.

Physical and chemical analyses of fraction 2B1B (Table IV) indicated that it was a dimer of methyl oleate joined by a carbon-to-carbon linkage with one carbonyl group in the molecule. Its IR spectrum showed no *trans* configuration. Its NMR spectrum showed the following proton signals: very weak aroung 4.8τ (double bond), 6.4τ (methyl ester), around 7.8τ (methylene group adjacent to ester group), 8.65τ (methylene group), around 9.0τ (terminal methyl group).

Characterization of the Polar Polymeric Fractions

Fraction 3B was separated by preparative thin layer chromatography on silica gel using benzene-ethyl ether (90:10) as the eluent (2) into two fractions: 3B1 (91.0%) and 3B2 (9.9%). Fraction 4B was similarly fractionated using ethyl ether-benzene (85:15) as the developer into 4B1 (78.1%) and 4B2 (21.9%). Further attempts to fractionate 3B1, 4B1, 5B, 6B, and 7B were unsuccessful.

In order to elucidate the chemical nature of the bond joining these polymers, fractions 3B1, 4B1, 5B, 6B, and 7B were each depolymerized with anhydrous methanol solution of hydrochloric acid. Molecular weight determination of each of the samples before and after the depolymerization indicated that the polymers remain unattacked. It is, therefore, evident that the polymers are not joined through peroxide linkages like those formed by autoxidation at low temperatures (8).

These fractions, on the other hand, were each depolymerized successfully with hydroiodic acid. The preparative thin layer chromatogram of each of these fractions and its depolymerized product using silica gel as an adsorbent and benzene as an eluent are shown in Figure 3.

The R_f value and amount of each of the three fractions obtained by thin layer chromatography of the depolymerized products of fractions 3B1, 4B1, 5B, 6B, and 7B are summarized in Table V. The fast moving fractions (3B1A, 4B1A, 5B1, 6B1, and 7B1) were monomers according to their molecular weights. The dimer fractions (3B1B, 4B1B, 5B2, 6B2, and 7B2) and the trimer fractions (3B1C, 4B1C, 5B3, 6B3, and 7B3) were analyzed, and the results are shown in Table VI. Comparison of the amount of oxygen in each fraction by elemental analysis and functional group analysis indicated that all fractions except 3B1 still contain oxygen atoms which could not be accounted for by the functional groups analyzed (Table VII).

From the data on depolymerization study (Tables V & VI), it can be concluded that triolein treated under simulated deep-fat frying conditions contained 1.1% trimers with carbon-to-carbon linkages, 1.9% dimers and trimers joined through carbon-to-carbon linkages, and 3.1% dimers and trimers joined through carbon-to-oxygen or carbon-to-carbon linkages in the same molecule and also dimers and trimers in which all the monomeric units were joined through carbon-to-oxygen linkage.

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REFERENCES

1. Artman, N.R., Adv. Lipid Res. 7:245 (1969).
2. Paulose, M.M., and S.S. Chang, JAOCS 50:147 (1973).
3. Hornstein, I., J.A. Alford, L.E. Eliott, and P.F. Crowe, Anal. Chem. 32:540 (1960).
4. Jamieson, G.R., and E.H. Reid, J. Chromatogr. 17:230 (1965).

5. Firestone, D., W. Horwitz, L. Friedman, and G.M. Shue, JAOCS 38:418 (1961).
6. Sahasrabudhe, M.R., and D.G. Chapman, Ibid. 38:88 (1961).
7. Paschke, R.F., L.E. Peterson, and D.H. Wheeler, Ibid. 41:723 (1964).
8. Chang, S.S., and F.A. Kummerow, Ibid. 30:403 (1953).
9. Williamson, L., J. Appl. Chem. 3:301 (1953).
10. "Official and Tentative Methods of the American Oil Chemists' Society," Third Edition, AOCS, Champaign, IL, 1964 (revised to 1972).
11. Mehlenbacher, V.C., "The Analysis of Fats and Oils," Garrard Press, Champaign, IL, 1960, pp. 307, 349, 490.
12. Bhalerao, V.R., J.G. Endres, and F.A. Kummerow, JAOCS 38:689 (1961).
13. Paschke, R.F., L.E. Peterson, S.A. Harrison, and D.H. Wheeler, Ibis. 41:56 (1964).
14. Gupta, A.K.S., Fette Seifen Anstrichm. 69:907 (1967).

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