Cyclic Fatty Acid Monomer Formation in Frying Fats. I. Determination and Structural Study

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The formation of monomeric cyclic fatty acids was studied in a model system in which partially hydrogenated soybean oil (PHSO) was heated intermittently for 80 hr of simulated deep fat frying. Oil samples (fresh and heated) and their methyl esters were fractionated according to their molecular size using gel permeation chromatography (GPC). Oils and GPC fractions were analyzed for cyclic monomers by the following steps: (i) preparation of fatty acid methyl esters (FAME); (ii) microhydrogenation of FAME; (iii) urea fractionation of hydrogenated FAME; (iv) analysis by capillary gas liquid chromatography (GLC), and (v) structural characterization of cyclic monomer peaks by mass spectrometry (GC-MS). Under simulated frying conditions the concentration of cyclic monomers increased from 736 ppm (0.07%) in fresh oil to 1803 ppm (0.18%) in heated oil. GC-MS with capillary columns allowed the identification of several C_{18} α-disubstituted cyclohexane and cyclopentane isomers as hydrogenated methyl esters. Other noncyclic and contaminant compounds eluting within the expected GLC retention range of cyclic monomers also were identified in all the samples and GPC fractions.

When fats and oils are used for deep fat frying operations, thermal and oxidative deterioration of the lipid components takes place, producing volatile and nonvolatile decomposition products (1-3). The extent and nature of these products are affected considerably by the characteristics of the fried food, the composition of the fat and the frying conditions (1,4,5): temperature, exposure to oxygen, heating period, frying capacity (kg food/hr), continuous or intermittent frying, mode of heat transfer, metals in contact with the oil, cleanliness of the fryer, turnover rate and initial quality of the fat. However, the accumulation of the degradation products in the frying medium and their eventual incorporation in the fried foods becomes of primary concern when commercial or industrial frying operations are carried out under abusive conditions.

The nonvolatile degradation products found in abused frying oils include polymeric triacylglycerols, many oxidized triacylglycerol derivatives, cyclic substances and some breakdown products (2,10). Polymeric triacylglycerols result from the condensation of two (dimer) or more triacylglycerol molecules to form high molecular weight compounds (polar and nonpolar) of more than 1,800 daltons. The nonpolymerized part of the oil contains mainly the unchanged triacylglycerols in combination with their oxidized derivatives. In addition, it contains lesser amounts of mono- and diacylglycerols, partial glycerides containing chain-scission products, triacylglycerol with cyclic and/or dimeric fatty acids and any other nonvolatile fragmentation products with molecular weights of less than 900 daltons. Upon methanolysis of all these lipid species, the products obtained can be arbitrarily classified as (i) polymeric fatty acid methyl esters with MW around 600 daltons or more (polar and non-polar), and (ii) monomeric fatty acid methyl esters which include unchanged, changed (oxidized, cyclized, isomerized, etc.) and fragmented fatty acid esters with molecular weights around 300 daltons or less.

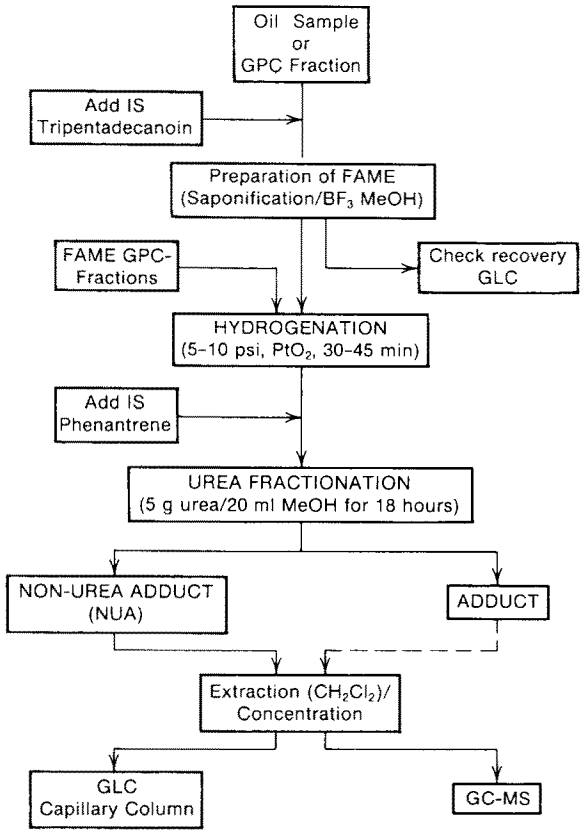

FIG. 1. Analytical steps for the quantitative determination of cyclic monomers. IS, internal standard.

Among the monomeric products, the presence of cyclic compounds originating from the intramolecular cyclization of the C_{18} polyunsaturated fatty acids is cause for concern from the nutritional toxicity point of view (7,8). It has been shown that incorporation of these compounds at 0.15% in low protein diets causes accumulation of liver lipids in rats (9). Furthermore, cyclic fatty acids (CFA) may be included in the body fat along with natural fatty acids because they are more readily absorbed by the digestive and lymphatic systems (6) when compared with dimeric and polymeric acids.

Several studies on thermal oxidation of vegetable oils have demonstrated the formation and/or increase of

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CFA monomers under conditions similar to frying (10-17). However, the structural characterization and quantification of all the cyclic isomers formed during deep fat frying is far from complete. Potteau et al. (17) reported that the relative yield of CFA isomers formed during thermal polymerization of linseed oil is 10 times larger than during thermal oxidation of the same oil, but the chemical identity of the C_{18} hydrogenated cyclic esters confirmed by GC-MS was shown to be the same in both equally degraded oils. Another structural study (12) proved that partially hydrogenated soybean oil used for frying potato slices contained two of the most abundant CFA previously reported for linseed oil, 2-n-propyl and 2-n-butyl cyclohexyl carboxylates. This and many other laboratory studies (13-16) showed that the levels of CFA in frying oils generally were below 0.5%, even though the identity of all the observed GLC peaks assigned to CFA and their relative concentration remained unclear.

Recently, two independent studies have reported the occurrence of monomeric cyclic fatty acids in fats and oils used for frying in commercial practice. Frankel et al. (18) showed that 0.1 to 0.7% of these compounds are present in several oil samples from USA and Middle East commercial frying processes. Gere et al. (19) reported cyclic monomers levels of 0.02 and 0.04% in lard samples and 0.03 to 0.16% in sunflower oil samples obtained from frying operations in Hungary. Although these authors used different analytical approaches to estimate the concentration of cyclic monomers, their results agreed with the levels predicted by laboratory

TABLE 1

Characteristics of PHSO Before and After Simulated Deep Fat Frying (80 hr @ 195 C)

Characteristics	Fresh oil	Heated oil
Iodine value	108.9	101.3
Saponification value	191.4	195.9
Free fatty acids, % (as oleic)	0.03	0.59
Hydroxyl value	2.25	9.34
Monoacylglycerols, % (by GLC)	ND	0.06
Diacylglycerols, % (by GLC)	1.18	2.73
Fatty acid composition (as FAME)[a]		
Myristic 14:0	0.06	0.06
Palmitic 16:0	9.90	9.82
Stearic 18:0	4.53	4.45
Oleic 18:1	45.3	42.9
Linoleic 18:2	37.0	29.6
Linolenic 18:3	2.39	1.67
Arachidic 20:0	0.35	0.35
Behenic 22:0	0.38	0.38
Others[b]	0.50	0.67
Total recovery (%)	100.41	89.90
NEM[c] (100 − % recovery)	0.00	10.10

ND = non detectable.
[a]g FAME/100 g oil.
[b]Not identified compounds.
[c]Non-elutable materials after GLC.

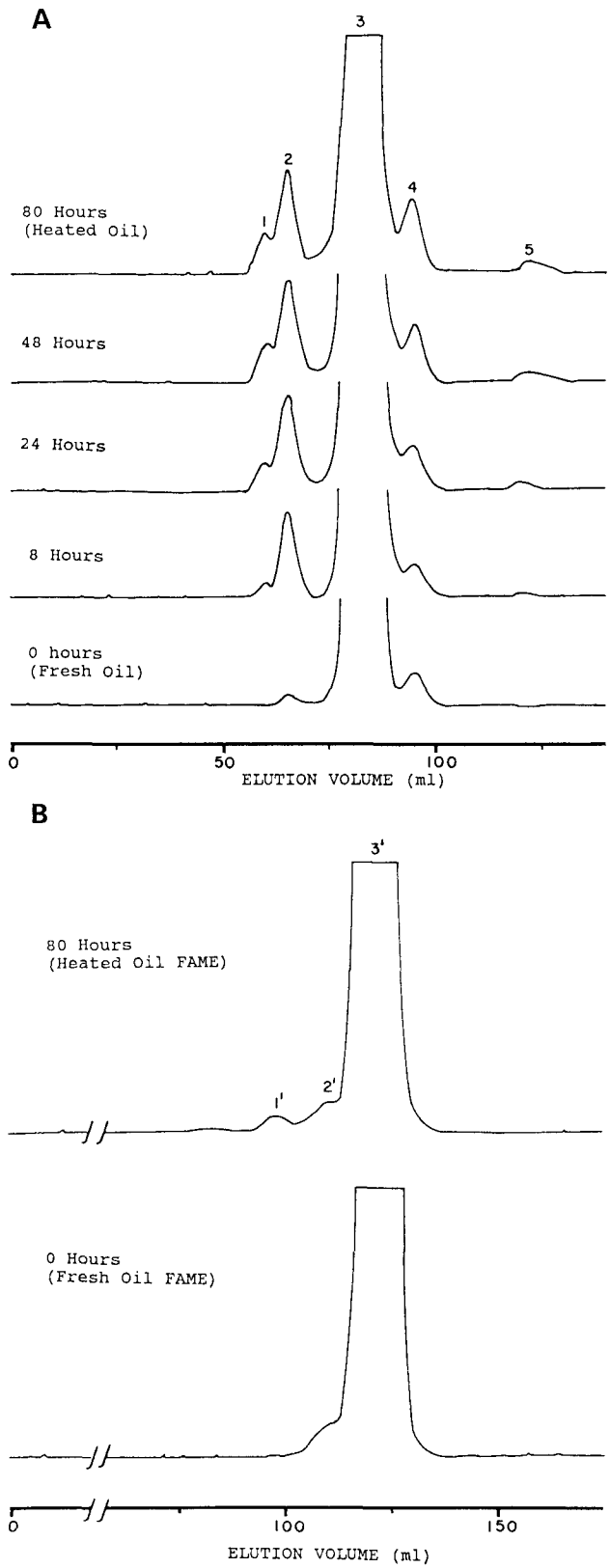


FIG. 2. Gel permeation chromatograms. A, PHSO samples during simulated deep fat frying (1, trimer triacylglycerols; 2, dimer triacylglycerols; 3, triacylglycerols; 4, diacylglycerols; 5, free fatty acids). B, Fatty acid methyl esters, (FAME's) (1′, dimer FAME's; 2′, unsaponifiables and high molecular weight FAME's; 3′, FAME's).

studies. These authors also suggested that the amount of CFA present can be correlated with heat abuse as well as the proportion of unsaturated fatty acids in the original fat.

The present study was carried out to determine the distribution of CFA between size excluded fractions of partially hydrogenated soybean oil used for simulated frying. Gel permeation chromatography was used for the preparation of the fractions. The individual quantification of CFA as well as their structural identification was carried out on hydrogenated methyl esters using high resolution capillary columns and GC-MS.

EXPERIMENTAL PROCEDURES

Materials. Partially hydrogenated soybean oil (PHSO) was donated by Humko Inc. (Memphis, Tennessee). All the solvents used during methylation, extractions and concentration steps were nanograde quality. However, toluene, n-hexane and methylene chloride were redistilled in glass to eliminate possible interferences after concentration. Platinum oxide (83.4% pure) was purchased from Englehard Industries, Inc. (Newark, New Jersey). Urea was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). Tripentadecanoin (99+% pure) was obtained from NuChek Prep. (Elysian, Minnesota) and phenanthrene from Supelco, Inc. (Bellefonte, Pennsylvania).

Simulated deep fat frying. The frying operation was carried out in a household appliance type deep fat fryer in which 2.5 l of PHSO were heated intermittently at 195±5 C for 80 hr (8 hr/day). Batches of 30 g of moist cotton balls containing 75% by weight of water were fried at 30-min intervals (17 frying operations/day). Cotton balls prewashed with hexane and then with

TABLE 2

Yields of GPC Fractions of Fresh and Heated PHSO (Oils and Methyl Esters)

GPC-fraction code	Peaks[a]	Molecular species[b]	$MW_{app.}$[c]	Fresh oil (%, w/w)[d]	Heated oil (%, w/w)[d]
I	1	Tri-TG	2650	1.1	15.5
	2	Di-TG	1760		
II	3	TG	880	96.0	79.6
III	4	DG	620	1.9	3.9
—	5	FFA	280	<1.0	<1.0
ME-I	1′	Di-FAME	600	1.7	6.6
ME-II	2′ & 3′	FAME	300	98.3	93.4

[a]Peak numbers according to Fig. 2 (A & B).
[b]Most important molecular species: Di, dimer; Tri, trimer; TG, triacylglycerols; DG, diacylglycerols: FFA, free fatty acid; FAME, fatty acid methyl esters.
[c]Approximate molecular weight.
[d]Weight percent averaged for 3 determinations (standard deviation = 0.8%).

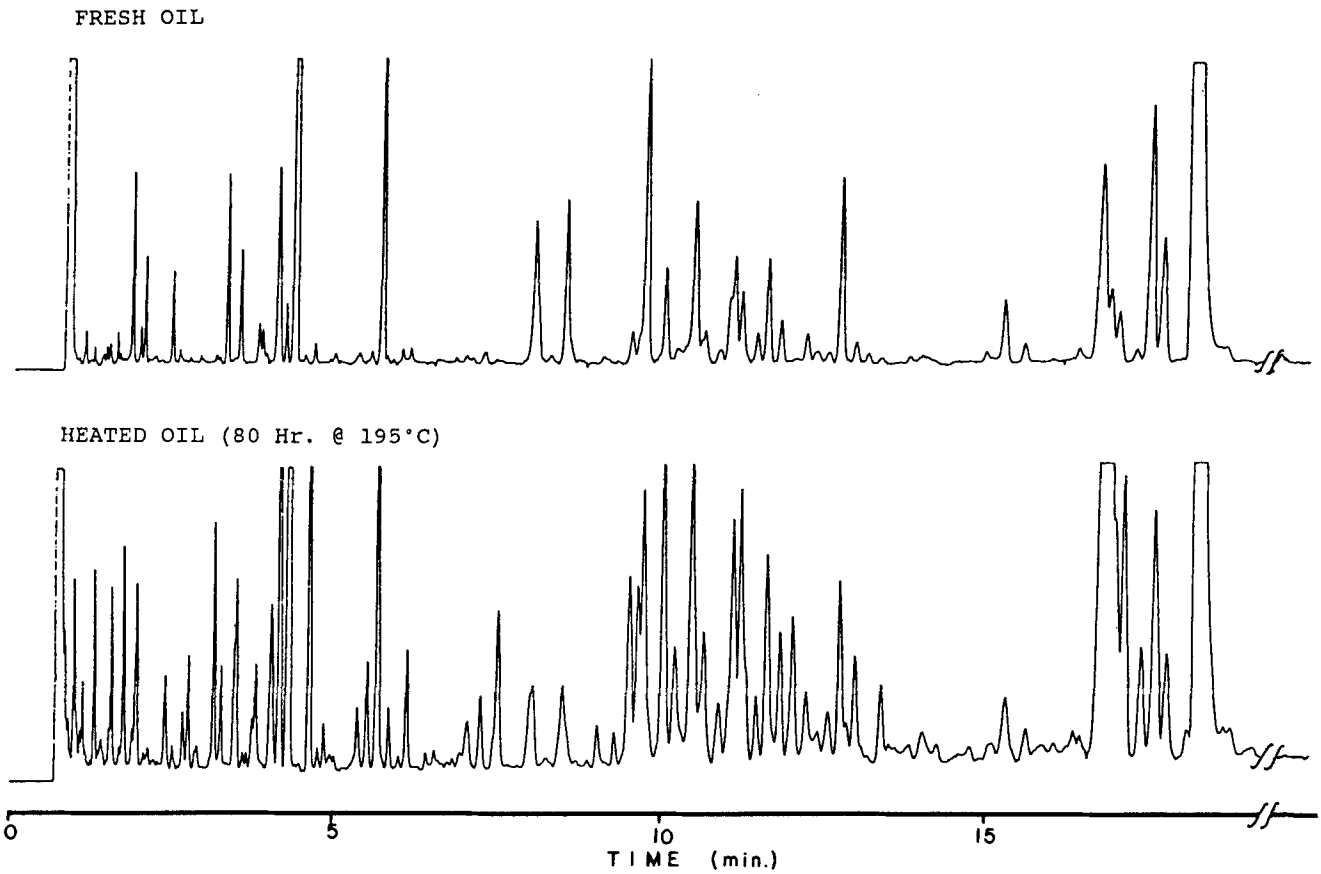


FIG. 3. Capillary GLC chromatograms of non-urea adducting (NUA) fractions.

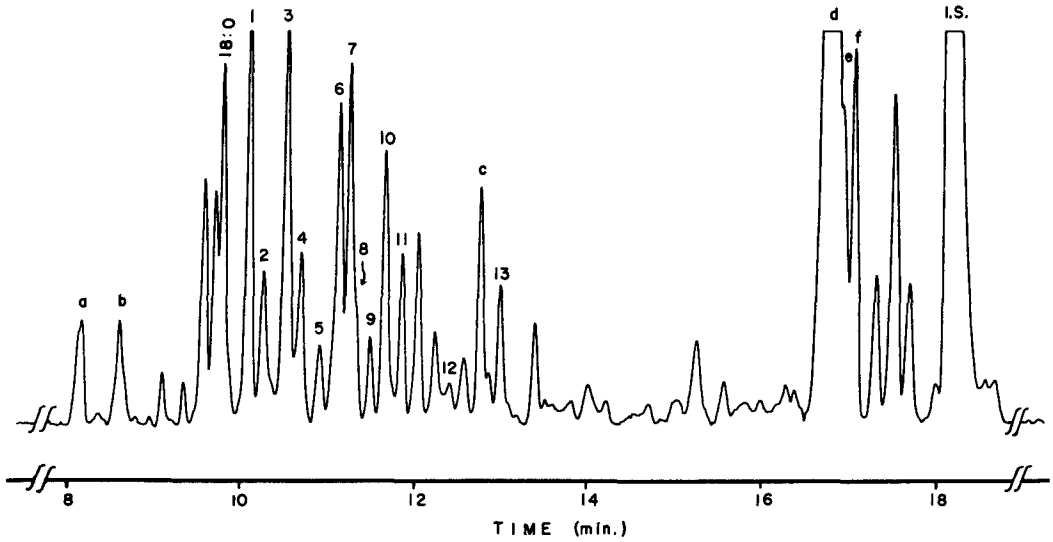


FIG. 4. Partial chromatogram of heated oil NUA-fraction showing peak codes used for the study. I.S., Internal standard—Phenanthrene (RRT = 1.845, ECL = 20.324).

ethanol were used as substitute for food to avoid the complication of the fried food components as previously suggested by Chang et al. (3). 100-ml samples were collected at the end of each 8-hr frying period, blanketed with nitrogen and stored in the freezer. The daily turnover rate of fresh oil averaged 24% through the whole operation. This was determined by the amount of oil needed to replace what was removed in sampling or absorbed by the cotton balls.

Chemical characterization. Fresh and heated oil samples were analyzed using AOCS official methods (20) for: iodine value (Cd 1-25), saponification value (Cd 3-25), free fatty acids (Ca 5a-40), hydroxyl value (Cd 13-60) and fatty acid composition (Ce 1-62). The amount of GLC nonelutable materials (NEM) was estimated by subtraction from 100 of the total fatty acid recovery plus any other non-identified GLC peak eluting during the GC run (tripentadecanoin was used as internal standard). The GLC method recently reported by Goh and Timms (21), with slight modifications, was used to analyze monoacylglycerols and diacylglycerols as their TMS derivatives.

Gel permeation chromatography (GPC). The separation of monomeric fractions via GPC was conducted in two glass columns (Cheminernt, LDC/Milton Roy) of 12.7 mm i.d. and 109 cm length connected in series and packed with Bio-Beads S-X2 (Bio-Rad, Richmond, California) swollen in toluene. A Tracor 995 isochromatographic pump (Tracor, Inc., Austin, Texas) delivered toluene, the mobile phase, at a constant flow rate of 1 ml/min. A Rheodyne 7120 sample injector (Rheodyne, Berkeley, California) with a 100-μl loop was used to deliver the oil samples into the column. A differential refractometer Waters Model R-403 (Waters Associates, Framingham, Massachusetts) continuously monitored the column effluent, and the signal was recorded with a Hewlett Packard electronic integrator HP 3390A (H.P., Avondale, Pennsylvania). Filtered samples of pure oil (heated and unheated) and methyl esters were injected as such directly into the system. Repeated injections were performed in order to obtain approximately 300-400 mg of monomeric fractions. The fractions were collected in tared flasks which were reweighed after evaporation of the solvent in a rotary evaporator (Buchler Instruments, Fort Lee, New Jersey). The flasks containing each fraction were blanketed with nitrogen gas and stored in the freezer until required.

Determination of cyclic monomers. The method used for the determination of CFA is based on the approaches proposed by Potteau et al. (17) and Gere et al. (15), with modifications. The procedure included preparation of fatty acid methyl esters, hydrogenation, urea fractionation-concentration and capillary GLC. The identity of the cyclic compounds was verified by GC-MS in all the samples. An illustration of the steps involved in the quantitative method is included in Figure 1.

Preparation of fatty acids: The AOCS official method Ce 2-66 (20) was used with tripentadecanoin as internal standard to check on the recovery of monomeric methyl esters. The fatty acid methyl esters (FAME) were extracted with n-hexane, dried over anhydrous sodium sulfate and the solvent evaporated in a rotary evaporator.

Hydrogenation: 30-120 mg FAME dissolved in 10 ml methanol were catalytically hydrogenated over 10-15 mg of platinum oxide using a microhydrogenator (Supelco, Inc., Bellefonte, Pennsylvania). The reaction mixture was stirred and kept under 5 psi of hydrogen pressure for 30 min. The contents were filtered through a fluoropor filter type FH, 0.5 μm pore size (Millipore Lab. Prod., Bedford, Massachusetts). The filtrate was collected in a tared teflon lined screw cap test tube and the solvent evaporated in a steam bath under a stream of nitrogen.

Urea fractionation-concentration: Hydrogenated fatty acid methyl esters (HFAME) were weighed accurately in a test tube, and an aliquot of 250 μl of phenanthrene solution (0.50 mg/ml) was added as an

TABLE 3

Concentration of Monomeric Cyclic Fatty Acids in Fresh and Heated Oil and their GPC Fractions

			Fresh oil[a]						Heated oil[a]							
	Retention data		Oil (alone)		II[b]		ME-II[b]		Oil (alone)		I[b]		II[b]		ME-II[b]	
Peak code[c]	RRT[d]	ECL[e]	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	1.033	18.121	80	10.9	98	12.2	78	11.7	273	15.1	317	16.5	251	17.9	264	15.5
2	1.048	18.180	18	2.4	25	3.1	<10	1.5	115	6.4	145	7.5	50	3.6	71	4.2
3	1.076	18.276	177	24.0	178	22.1	135	20.3	296	16.4	314	16.3	186	13.3	290	17.1
4	1.095	18.342	39	5.3	12	1.5	41	6.2	130	7.2	209	10.9	53	3.8	136	8.0
5	1.119	18.425	19	2.6	<10	1.2	20	3.0	54	3.0	59	3.1	48	3.4	50	2.9
6	1.140	18.495	149	20.2	180	22.4	131	19.7	230	12.8	193	10.0	224	16.0	219	12.9
7&8	1.152	18.535	67	9.1	79	9.8	75	11.3	268	14.9	299	15.5	244	17.4	262	15.4
9	1.178	18.620	27	3.7	35	4.4	22	3.3	55	3.1	42	2.2	<10	0.7	47	2.8
10	1.194	18.671	91	12.4	113	14.1	85	12.8	169	9.4	98	5.1	140	10.0	133	7.8
11	1.216	18.740	37	5.0	53	6.6	38	5.7	103	5.7	134	7.0	100	7.1	104	6.1
12	1.275	18.940	12	1.6	<10	1.2	<10	1.5	30	1.7	37	1.9	<10	0.7	39	2.3
13	1.333	19.089	20	2.7	11	1.4	19	2.9	80	4.4	79	4.1	84	6.0	83	4.9
Total cyclic acids			736	100	804	100	664	100	1803	100	1926	100	1400	100	1698	100

[a]Nonurea-adducting-fractions (NUA).
[b]GPC fractions; codes according to Table 2.
[c]Peak codes according to Fig. 4.
[d]Relative retention time to methyl stearate (RRT = 1.000).
[e]Equivalent chain length.
A, Concentration in parts per million (ppm), w/w in the hydrogenated sample averaged for two determinations (sd = 10-15 ppm).
Note: values below 10 ppm are considered as 10 for the estimation of total cyclic acids.
B, Relative percent referred to total cyclic monomers.

internal standard. Boiling methanol (20 ml) containing 5 g of urea was added to the test tube; the tube was then capped and thoroughly shaken until some crystals started to form. The crystalline suspension was allowed to stand from 18 hr in the dark. The urea crystals were filtered through Whatman #2 folded filter paper and washed with three portions of methanol saturated with urea. The filtrate and the washings were collected in a 100-ml separatory funnel and extracted three times with 5-ml portions of methylene chloride. The combined organic extracts containing the nonurea adduct (NUA) forming HFAME were washed twice with 10 ml of distilled water and finally dried over anhydrous Na_2SO_4. The NUA extract was concentrated under a stream of nitrogen to 0.1-0.5 ml, depending on the initial weight of HFAME.

Capillary gas chromatography. The concentrated NUA fractions were injected (1-3 μl) to a Hewlett Packard 5792 A capillary gas chromatograph (Hewlett Packard, Avondale, Pennsylvania) equipped with all-glass inlet splitter system, flame ionization detector and electronic integrator HP 3390A. A 30 m × 0.25 mm i.d. fused silica WCOT column coated with Supelcowax-10 (polyethylene glycol bonded phase), with a film thickness of 0.15 μm, (Supelco, Inc., Bellefonte, Pennsylvania), was used under the following conditions: column pressure, 15 psi; split ratio, 1:100; hydrogen as carrier gas; oven temperature programmed from 175 C to 200 C at 1.5 C/min and kept constant at 200 C for 5 min. The injector port and detector temperatures were 230 and 250 C, respectively.

Gas chromatography-mass spectrometry (GC-MS). Mass spectra of cyclic monomers were determined with a Hewlett Packard 5985B GC-MS (Hewlett Packard, Avondale, Pennsylvania) with the CI-EI source set at 70 ev and 200 C. The gas chromatograph was equipped with the same column described above, and the oven was operated with a multi-ramp temperature program from 190 to 205 C at 1.5 C/min and then raised to 250 C at the rate of 5 C/min; the carrier gas was helium with a split ratio of 1:100 and inlet column pressure of 10 psi; injector temperature, 230 C; for chemical ionization (CI) mode, methane was used as the reactant gas which entered the MS source via the jet separator.

RESULTS AND DISCUSSION

The conditions for the frying operations were selected to simulate those used in commercial deep fat frying. The use of moist cotton balls instead of food allowed those reactions which are promoted by frying to take place within the oil, avoiding the interference of food constituents and their eventual heat alteration and leaching.

The absolute changes in the chemical constants of PHSO after 80 hr of frying can be observed in Table 1. As expected, a net decrease in unsaturation was evidenced by the lower iodine values of the heated oil and by the significant decrease in oleic and polyunsaturated fatty acids. Similarly, the increase in free fatty acid level indicates hydrolytic reactions taking place during frying, accompanied by the liberation of

TABLE 4

C_{18} Cyclic Monomers Identified by GC-MS

Peak code[a]	n(m)[b]	Main component	Configuration
1	4(1)	methyl-9-(2′-n-butylcyclopentyl)-nonanoate	*trans-*
2	5(2)	methyl-7-(2′-n-pentylcyclohexyl)-heptanoate	*trans-*
3	3(1)	methyl-10-(2′-n-propylcyclopentyl)-decanoate	*trans-*
4	4(2)	methyl-8-(2′-n-butylcyclohexyl)-octanoate	*trans-*
5	5(2)	methyl-7-(2′-n-pentylcyclohexyl)-heptanoate	*cis-*
6	4(1)	methyl-9-(2′-n-butylcyclopentyl)-nonanoate	*cis-*
7 & 8	3(2)	methyl-9-(2′-n-propylcyclohexyl)-nonanoate	*trans-*
	4(2)	methyl-8-(2′-n-butylcyclohexyl)-octanoate	*cis-*
10	3(1)	methyl-10-(2′-n-propylcyclopentyl)-decanoate	*cis-*
11	3(2)	methyl-9-(2′-n-propylcyclohexyl)-nonanoate	*cis-*
12	2(2)	methyl-10-(2′-n-ethylcyclohexyl)-decanoate	*trans-*
13	2(2)	methyl-10-(2′-n-ethylcyclohexyl)-decanoate	*cis-*

[a]Peak codes according to Fig. 4.
[b]n(m) = value of n in the general formula for α-dissubtituted cyclohexane (m = 2) or α-disubstituted cyclopentane (m = 1).

diacylglycerols and monoacylglycerols, as confirmed by the GLC results included in the same table. The amount of nonelutable oxidized and polymerized products reached 10.1 g/100 g of oil after 80 hr of intermittent frying.

Fresh and heated oils and their methyl esters were separated into fractions according to molecular size, using GPC. The GPC chromatograms for selected oil samples at different heating periods during the simulated frying are shown in Figure 2 (A and B). Fresh oil (0 hr in Fig. 2A) contained a small amount of dimerized triacylglycerols probably formed during oil processing due to the high temperatures achieved in the deodorization stage. After 8 hr of frying the proportion of the dimer peak increased significantly, and a small peak of trimer triacylglycerols was observed (peak 1 in Fig. 2A). The amounts of trimer and dimer, estimated as peak sizes, continued to rise until the end of the heating period, but after 48 hr only slight changes were observed. Figure 2B shows the GPC chromatograms resulting when FAME are separated by molecular size. The peak of polymeric FAME (peak 1′, Fig. 2B) however, becomes apparent after a few days of frying.

The weight proportion of the collected fraction during GPC is reported in Table 2. A highly significant increase in polymeric triacylglycerols (Fraction I) and diacylglycerols (Fraction III) which parallel a proportional decrease in triacylglycerols species (Fraction II) upon frying is clearly indicated. However, the amounts of diacylglycerol in Table 2 for fresh and heated oil showed higher values compared with the results from GLC (Table 1); this can be explained by incomplete resolution of the diacylglycerol peak by GPC as well as the higher relative error associated with small amounts of fractions. After methanolysis, the yield of polymeric FAME (Fraction ME-I) increased almost fourfold after 80 hr of simulated frying. However, this increase cannot be correlated with the peak sizes in the GPC chromatograms in Figure 2B. This probably is due to similarities in refractive index between the mobile phase, toluene and the dimer fatty acids present in the sample.

All the fractions which were obtained either by GPC of oil alone or by GPC of FAME, and which contained significant amounts of monomeric compounds, were analyzed for cyclic acid after hydrogenation followed by urea fractionation-concentration. The basic approach of Potteau et al. (17) modified by Gere et al. (15) was selected as the most convenient procedure for the quantitative determination of cyclic monomers. However, further modifications of the methylation procedure, and the conditions for hydrogenation, urea fractionation and GLC were made. Figure 1 shows schematically all the steps included in the modified analytical procedure.

The partial chromatograms obtained from the NUA fractions prepared for fresh and heated oil are included in Figure 3. The last part of the chromatograms (elution time > 20 min), not shown in Figure 3, contained many nonidentified peaks, especially for heated oil and their fractions. It was necessary to increase the column temperature close to the limit temperature of the stationary phase in order to hasten the elution of the high molecular weight and other retained components present in the samples.

The portion of the chromatogram of heated oil NUA fraction containing the cyclic monomers is shown in Figure 4. The peak identification code numbers and letters used for the present study also are included in this figure. The peaks identified with a number were confirmed to be C_{18} cyclic methyl esters by GC-MS, whereas those identified with letters were positively recognized chemical compounds.

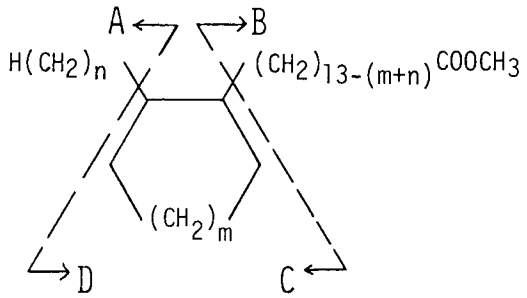
The amounts of individual cyclic monomers in the samples, as well as fractions analyzed, are included in Table 3. The retention data averaged for six determinations also is included in this table as retention ratio to methyl stearate and as equivalent chain length (ECL) calculated according to Miwa et al. (22) under the experimental conditions used.

It can be observed in Table 3 that there is a 2.5-fold

increase in the total amount of cyclic acids during the frying operation, from 736 ppm in the fresh oil to 1803 ppm in heated oil. Approximately the same level of increase is also observed for total cyclics when the monomeric Fractions ME-II for fresh and heated oil are compared. In contrast, the total cyclic monomer concentration for Fraction II of fresh oil showed only a two-fold increase when compared with the equivalent fraction of fresh oil. In addition, the amount of cyclic acids in GPC Fraction II of heated oil was around 400 ppm less than in the heated oil alone.

The last two observations suggest that other GPC fractions of the heated oil should contain higher proportions of cyclic acids in order to balance their total amount present in the heated oil itself. This was indeed demonstrated when Fraction I of heated oil was analyzed for cyclic acids. As shown in Table 3, the amount of cyclic C_{18} acids in Fraction I was more than 500 ppm larger than that in Fraction II from heated oil. Furthermore, the cyclic monomers corresponding to peaks 1 through 4 in Fraction I are mainly responsible for this higher concentration.

Chemical ionization (CI) and electron impact (EI) mass spectra were obtained for all the eluting peaks suspected to be C_{18} cyclic monomers. The expected EI spectra of methyl esters of saturated cyclic acids can be derived from the theoretical fragmentation pattern of α-disubstituted cycloalkanes with one of the substituent groups being an n-alkyl chain and the other a linear methyl carboxylate moiety. The following general formula shows the expected fragments for C_{18} cyclic methyl ester with five or six carbon membered rings:



where m = 1 is for cyclopentyl isomers, m = 2 for cyclohexyl isomers and n can take all the possible values to get 18 carbons in the acid chain, that is $1 < n < (13-m)$.

For each structural isomer (one set of values of n and m) four characteristic ion fragments (A, B, C and D) are expected in relatively high abundance. Additional ion fragments can result from loss of methanol by α-cleavage of fragment D (D-32) followed by loss of water (D-32-18) and from the protonation of fragment B (B+1).

The structures reported in Table 4 were assigned to each peak of Figure 4 after study of their individual MS fragmentation patterns. Nevertheless, some complications became apparent when matching total ions (TI) plots obtained during GC-MS with the chromatograms obtained by capillary GC with FID detector like the one shown in Figure 4: the shoulder identified as peak 8 in Figure 4 was not observed, and peak 9 appeared as a small blip (M^+ at m/e 296) not resolved from peak 10, even though both peaks (8 and 9) were present in all the samples. This was attributed to common resolution losses experimented in our system when connecting capillary columns to the GC-MS valve.

The mass spectrum of the coeluting peaks 7 and 8 is shown in Figure 5. All the characteristic ion fragments of methyl-9-(2′-n-propylcyclohexyl) nonanoate (m=2, n=3 in the general formula above) are present in this spectra at significant abundances. However, close examination of the same spectra also shows that the most important fragments of methyl-8-(2′-n-butylcyclohexyl) octanoate (m=2, n=4) are also present with lower intensities, namely the ions at m/e 139 (ion C), m/e 157 (ion B), m/e 158 (ion B+1), m/e 239 (ion D), m/e 207 (ion D-32), and m/e 189 (ion D-32-18). These observations lead us to conclude that under the conditions used the nonanoate is present in higher concentration than its coeluting octanoate, so the latter can be assigned to peak 8 as shown in Table 4.

Isomers of 2′-n-butyl (peaks 1 and 6) and 2′-n-propyl (peaks 3 and 10) cyclopentyl C_{18} monomeric esters were identified in all samples. The percent of these compounds (column B in Table 3) accounts for more than 50% referred to total cyclic monomers in all the samples except for GPC Fraction I of heated oil. Based on similar studies on thermoxidized linseed oil (17), it was expected that monomers containing cyclopentane rings would occur to a lesser extent. To verify this finding, further studies are being carried out in our lab in other oils and fats used for frying.

The configurations reported in Table 4 tentatively have been assigned in those cases in which two peaks give the same mass spectra. The *trans*-configuration was assigned to the earlier eluting cyclic isomer (lower RRT and ECL) in all cases, based on previous studies (23,24) that showed this trend when separating chemically synthesized cyclic monomers by GLC. However, further studies are necessary to confirm this elution pattern under our experimental conditions, especially for the disubstituted cyclopentane isomers.

Other noncyclic compounds, also identified by GC-MS, are included in Table 5. Peaks *a* and *b* (Fig. 4)

TABLE 5

Other Compounds Identified by GC-MS

Peak code[a]	ECL[b]	Main components
a	17.290	branched chain C_{18} fatty acid methyl ester
b	17.503	branched chain C_{18} fatty acid methyl ester
c	19.203	butyl phthalate (solvent contaminant)
d & e	20.036	methyl-9-methoxy-stearate
	20.072	methyl-10-methoxy-stearate
f	20.102	methyl-12-methoxy-stearate methyl-13-methoxy-stearate

[a]Peak codes according to Fig. 4.
[b]ECL, Equivalent chain length.

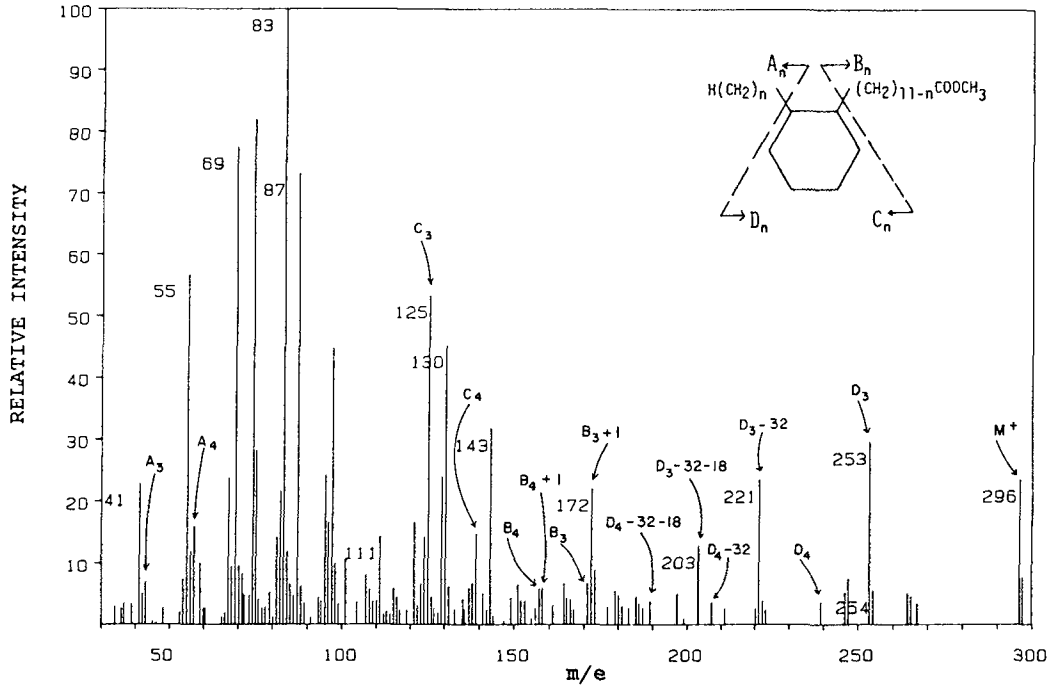


FIG. 5. Mass spectra of coeluting peaks 7 and 8. n = 3 for methyl-9-(2′-n-propylcyclohexyl)-nonanoate; n = 4 for methyl-8-2′-n-butylcyclohexyl)-octanoate.

both have molecular ion at m/e 298, and appear to correspond to several branched chain isomers of methyl stearate previously reported in oxidized soybean oil by Artman and Alexander (11). Peak *c* (Fig. 4) was identified as dibutyl phthalate, a contaminant present in the n-hexane used in our extractions, which was not totally removed during distillation. Peaks *d*, *c* and *f* showed mass spectra analogous to those reported by McCloskey (25) and Potteau et al. (17) for monomethoxy methyl stearate isomers, which may have been formed during the preparation of methyl esters.

Given the diversity of all possible interferences during the analysis of cyclic monomers in oils, our results show the need to introduce a confirmatory step, like mass spectrometry, in all proposed methods for their determination. On the other hand, the complex chemical nature of the nonurea adducting fraction (NUA) in which all the cyclics are isolated, suggests either the introduction of an additional step for the purification of the NUA fraction, or the development of new analytical approaches using novel techniques such as HPLC, supercritical fluid chromatography or multidimensional GC.

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