Heated Fat-Based Oil Substitutes, Oleic and Linoleic Acid-Esterified Propoxylated Glycerol

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ABSTRACT: Four oils [triolein, trilinolein, oleic acid-esterified propoxylated glycerol (EPG-08 oleate), and linoleic acid-esterified propoxylated glycerol (EPG-08 linoleate)], each without added antioxidants, were heated for 12 h/d at approximately 190°C in a small deep-fat fryer until the polymer concentration exceeded 20%, as determined by high-performance size-exclusion chromatography. Increases in the free fatty acid content, total acid value, food oil sensor value, and *p*-anisidine value during heating indicated that significant thermal oxidation had occurred in each oil. Capillary supercritical fluid chromatography (SFC) was used to determine the substrate concentration of each oil after each heating interval. The average, apparent first-order reaction rate constant (as determined by SFC) for trilinolein was 0.0348 ± 0.0034 h^{-1}, while the rate for EPG-08 linoleate was 0.0253 ± 0.0032 h^{-1}. The average apparent reaction rate constant for triolein was 0.0256 ± 0.0011 h^{-1}, while the rate for EPG-08 oleate was 0.0252 ± 0.0008 h^{-1}. Triolein contained >20% polymer after 60 h of heating, EPG-08 oleate contained >20% polymer after 36 h of heating, and both trilinolein and EPG-08 linoleate contained >20% polymer after 24 h of heating.

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Fat substitutes can be divided into three broad categories: carbohydrate-based, protein-based, and fat-based (1). While many of the carbohydrate- and protein-based fat substitutes have received or would probably receive U.S. Food and Drug Administration (FDA) approval for food use with minimal testing, most of the fat-based fat substitutes will probably require extensive testing prior to approval. Because the carbohydrate- and protein-based fat substitutes developed to date cannot be used for frying applications, substantial commercial effort has been focused on the heat-stable, low-to-noncaloric, fat-based fat substitutes, in anticipation that they will eventually receive FDA approval. Fat-based fat substitutes are unique in that they can contribute little to the caloric content of the food, yet they retain the important functional attributes associated with "regular" fat. The widespread use of fat-based fat substitutes could result in a substantial reduction in the percentage of fat calories in the U.S. diet, which would have important positive implications in terms of heart disease and other cardiovascular problems.

Numerous fat-based fat substitutes have been developed or are under development (1). This report deals with the analysis of heated fatty acid-esterified propoxylated glycerols (EPG) (ARCO Chemical Company, Newtown Square, PA) and model triacylglycerols (TAG). To produce EPG, glycerol is propoxylated with propylene oxide to form a polyether polyol, which is then esterified with fatty acids (2–7). Preparation of fatty acid-esterified propoxylated glycerides for use as fat substitutes involves transesterifying propoxylated glycerol with fatty acid esters in a solvent-free, nonsaponifying system to avoid reagents that are considered unacceptable in food systems. After transesterification, the resultant fatty acid EPG contain a polyether extension between the fatty acid and glycerol that is resistant to lipase hydrolysis. The *in-vivo* threshold for nondigestibility occurs when the propoxylation number is ≥4. The preferred fatty acids are in the C_{14}–C_{18} range and could be derived from several sources, including soybean, olive, cottonseed, corn, milk fat, tallow, and lard. Fatty acid EPG are low- to noncaloric, only slightly digestible, with a heat stability comparable to TAG with similar fatty acid compositions. They have been substituted for fats and oils in table spreads, ice cream, frozen desserts, salad dressings, bakery products, salad oils, cooking oils, and shortenings. Most importantly, feeding studies of fatty acid EPG have indicated no toxicity.

Classical oxidation analyses and high-performance size-exclusion chromatography (HPSEC) (8,9) have been used to monitor secondary oxidation products in heated fats and oils, such as free fatty acids (FFA), aldehydes, and polymers. However, an assay that measures a single oxidation product or a limited number of oxidation products may not reflect the oil stability as accurately as an analysis that measures substrate concentration directly. The conditions under which oxidation occurs, the constituent fatty acids, and the presence or

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absence of antioxidants can alter the oxidation products, as well as the relative amounts of the various oxidation products. To determine oil stability, quantitation either of all of the oxidation products or of the substrate should be the most accurate. Capillary supercritical fluid chromatography (SFC) can be used to directly quantitate individual TAG substrate components (10), including the modified TAG that constitute fatty acid-esterified propoxylated glycerol.

To monitor the effects of heating at deep-fat frying temperatures on the stability of modified TAG (fatty acid-esterified propoxylated glycerol, EPG-08 oleate, and EPG-08 linoleate) and closely related TAG (triolein and trilinolein), a series of heating experiments were completed. Samples of each oil were separated with capillary SFC and HPSEC after heating. The objectives were: (i) to determine the amount of thermal oxidation that occurred upon heating each oil sample, (ii) to monitor the change in substrate concentration that occurred during heating; and (iii) to determine the apparent average rate of loss for EPG-08 oleate, EPG-08 linoleate, triolein, and trilinolein after intermittent heating at deep-fat frying temperatures.

EXPERIMENTAL PROCEDURES

Oil sample preparation. Oleic acid-esterified propoxylated glycerol with an initial reaction mixture mole ratio of 8 for propylene oxide to glycerol (EPG-08 oleate), linoleic acid-esterified propoxylated glycerol with a propylene oxide to glycerol mole ratio of 8 (EPG-08 linoleate), triolein, and trilinolein were obtained from ARCO Chemical Co. Each oil was synthesized with purified linoleic acid or oleic acid (approximately 92–93% for linoleic acid and >99% for oleic acid, as determined by capillary gas chromatography). Approximately 3.8 L of each oil, without added antioxidants, was heated in a small deep-fat fryer (Model F175A; Intedge Industries, Inc., Whippany, NJ) at approximately 190°C for 12 h/d until the TAG polymer concentration had exceeded 20% (10). Immediately after each 12-h heating period, the fryer was turned off, and the oil was allowed to cool to room temperature. At approximately 90–95°C, an oil sample of approximately 100–150 mL was removed from the fryer and placed in a dark amber glass bottle, blanketed with nitrogen, capped, sealed, and stored in a refrigerator (at approximately 2–5°C) until further analysis. The remaining oil and fryer were loosely covered with aluminum foil until the next 12-h heating period. Oil analyses were started the next morning and completed as soon as possible thereafter (10).

HPSEC. The HPSEC system consisted of an HP solvent delivery pump (Rainin Instrument Co., Woburn, MA), electronic pressure module, dual-chamber Dynamax dynamic mixer, prime-purge valve, 7030 Rheodyne (Coati, CA) switching valve, 7125 Rheodyne injection valve with 20-µL sample loop and 7161 Rheodyne position sensing switch, Phenogel 5 guard column (50 × 7.8 mm) (Phenomenex, Torrance, CA), followed by two Phenogel (5-µm particle size, 500 Å and 100 Å pore size) columns (500 × 8.0 mm) in series connected to an evaporative light-scattering detector (ELSD IIA; Varex Corp., Burtonsville, MD).

The mobile phase was tetrahydrofuran (THF, 1.0 mL/min). THF (Optima; Fisher Scientific, Fair Lawn, NJ) was filtered with 0.45-µm pore size HV discs (Millipore Corp., Bedford, MA), and degassed before use. THF was kept under a constant nitrogen gas purge while in use; no BHT was added as an antioxidant.

The ELSD IIA was used under the following conditions: adjusted temperature 100°C, heater temperature 98.3°C, exhaust temperature 59.5°C, gas flow rate 39 mm (top of ball in tube), pressure 11 psi, range 20, and time constant 0.5. Nitrogen gas of ultra-high purity (99.999%) was used.

Oil samples were prepared for HPSEC analysis by dissolving 30 µL of oil sample in 2970 µL THF (1:100 dilution) and then filtering the sample with a Millipore HV disc (0.45-µm pore size). Peak areas were determined with the Dynamax Method Manager Software, Version 1.3.1 (Rainin Instrument Co., Woburn, MA). All analyses were conducted in triplicate, and standard deviations were determined.

The molecular weight (MW) of the monomeric and polymeric components in the heated oil samples were estimated based on the retention volume (V_r). The response of the Varex detector was not linear with respect to MW. The response factor (R_f) was determined by plotting the area/concentration ratio vs. the MW of the standards (10).

The FFA value, acid value (AV), *p*-anisidine value (*p*-AV), peroxide value (PV), and food oil sensor (FOS) analysis were done as previously reported (10,11).

Statistical analysis. Statgraphics (Statistical Graphics Corp., STSC, Inc., Rockville, MD) was used to perform analysis of variance analysis with least-squares determination as the range test on all classical oxidation analyses (Tables 1–4).

Sample preparation for SFC. The internal standard was tridecylglycerol (Nu-Chek-Prep, Inc., Elysian, MN), and the sample solvent was methylene chloride (Fisher Scientific). Triolein and trilinolein samples were accurately weighed to ±0.00001 g and prepared at a concentration of approximately 2 mg/mL. The internal standard was accurately weighed and prepared at a concentration of approximately 1 mg/mL. The internal standard was mixed with the oil sample; the sample was diluted to approximately 0.4 mg/mL, and the internal standard was diluted to approximately 0.2 mg/mL. EPG-08 oleate and EPG-08 linoleate samples were accurately weighed to ±0.00001 g and prepared at a concentration of approximately 50 mg/mL. The internal standard was mixed with the oil sample; the sample was diluted to approximately 25 mg/mL, and the internal standard was diluted to approximately 0.2 mg/mL. Each sample was filtered through a 0.45-µm pore size HV disc (Millipore Corp.).

SFC apparatus. The supercritical fluid chromatograph was a Lee Scientific Model β501 (Lee Scientific, Inc., Div. Dionex, Salt Lake City, UT) and a Valco A90 injector (Houston, TX) with a 0.2-µL internal loop operated in a timed-split mode. Injection time was 0.05 s for the EPG-08 linoleate and

TABLE 1
Oil Analyses[a] of EPG-08 Oleate Heated at 190°C (12 h/d)

Oil analysis	Day 0 (0 h)	Day 1 (12 h)	Day 2 (24 h)	Day 3 (36 h)
Free fatty acid value[b]	0.25 ± 0.02[a]	0.81 ± 0.02[b]	1.07 ± 0.05[c]	1.33 ± 0.06[d]
Acid value[c]	0.54 ± 0.06[a]	1.40 ± 0.00[b]	2.02 ± 0.24[c]	2.09 ± 0.47[c]
p-Anisidine value[d]	3.2 ± 0.8[a]	54.8 ± 1.0[b]	65.4 ± 4.4[c]	67.7 ± 0.7[c]
Peroxide value[e]	9.2 ± 0.3[a]	1.5 ± 0.1[b]	0.4 ± 0.0[c]	0.0 ± 0.0[d]
Food oil sensor[f]	0.00 ± 0.00[a]	3.55 ± 0.04[b]	6.05 ± 0.05[c]	8.54 ± 0.01[d]
% Polymer	0.2 ± 0.0[a]	5.6 ± 0.2[b]	13.6 ± 0.4[c]	22.3 ± 0.2[d]

[a]Average of triplicates ± standard deviation. Values with the same letter (a,b,c,d) are not significantly different from each other at the 5% level for the same analysis.
[b]Units expressed as percentage oleic acid.
[c]Units expressed as mg KOH/g sample.
[d]Expressed as 100 times the optical density measured at 350 nm in a 1-cm cell of a solution (1.00 g oil/100 mL of solvent plus reagent).
[e]Units expressed as milliequivalents peroxide/1000 g of sample.
[f]Expressed as the ratio of polar to nonpolar components based on the dielectric constant; EPG-08 (ARCO Chemical Co., Newtown Square, PA).

TABLE 2
Oil Analyses[a] of Triolein Heated at 190°C (12 h/d)

Oil analysis	Day 0 (0 h)	Day 1 (12 h)	Day 2 (24 h)	Day 3 (36 h)	Day 4 (48 h)	Day 5 (60 h)
Free fatty acid value[b]	0.09 ± 0.02[a]	0.34 ± 0.04[b]	0.39 ± 0.06[b,c]	0.43 ± 0.02[c]	0.44 ± 0.04[c]	0.61 ± 0.05[d]
Acid value[b]	0.12 ± 0.00[a]	0.54 ± 0.06[b]	0.73 ± 0.06[c]	0.93 ± 0.00[d]	1.11 ± 0.06[e]	1.39 ± 0.00[f]
p-Anisidine value[b]	2.1 ± 0.4[a]	48.3 ± 0.3[b]	72.8 ± 0.4[b]	87.9 ± 1.5[c]	97.9 ± 2.4[d]	98.3 ± 2.2[d]
Peroxide value[b]	2.89 ± 0.11[a]	1.28 ± 0.13[b]	0.33 ± 0.12[c]	0.20 ± 0.10[c]	0.00 ± 0.00[d]	0.00 ± 0.00[d]
Food oil sensor[b]	0.00 ± 0.00[a]	2.15 ± 0.02[b]	3.60 ± 0.04[c]	5.21 ± 0.03[d]	6.71 ± 0.01[e]	8.44 ± 0.04[f]
% Polymer	0.0 ± 0.0[a]	2.3 ± 0.1[b]	6.6 ± 0.2[c]	11.4 ± 0.2[d]	17.4 ± 0.2[e]	26.5 ± 0.4[f]

[a]Average of triplicates ± standard deviation. Values with the same letter (a,b,c,d,e,f) are not significantly different from each other at the 5% level for the same analysis.
[b]See Table 1 for units.

EPG-08 oleate samples, while the injection time was 0.10 s for triolein and trilinolein. The capillary column used for the triolein and trilinolein separations was a 17-m SB-cyano-25 (50 µm i.d., d_f = 0.25 µm), with a stationary phase of 25% cyanopropyl, 25% phenyl, and 50% polymethyl siloxane, and a mobile phase of SFC-grade CO_2 (Scott Specialty Gases, Inc., Plumsteadville, PA). The separations were achieved by asymptotic density programming (0.2 g/mL, asymptotic ramp to 0.6 g/mL, 1/2 rise time 15 min, end time 60 min) at a column temperature of 100°C. The FID temperature was 375°C. The capillary column used for the EPG-08 oleate and EPG-08 linoleate separations was a 20-m SB-methyl-100 (50 µm i.d., d_f = 0.25 µm). The stationary phase in the column is 100% polymethyl siloxane. Asymptotic density programming (0.15 g/mL, asymptotic ramp to 0.64 g/mL, 1/2 rise time 15 min, end time 90 min) with a column temperature of 150°C was used for the EPG-08 linoleate separation. The oven, pump, and injector were controlled by an ARC Turbo PC (American

TABLE 3
Oil Analyses[a] of EPG-08 Linoleate Heated at 190°C (12 h/d)

Oil analysis	Day 0 (0 h)	Day 1 (12 h)	Day 2 (24 h)
Free fatty acid value[b]	0.25 ± 0.01[g]	0.45 ± 0.02[h]	0.85 ± 0.01[i]
Acid value[c]	0.23 ± 0.00[g]	0.54 ± 0.03[h]	1.31 ± 0.16[i]
p-Anisidine value[d]	3.1 ± 0.1[g]	167.0 ± 2.2[h]	156.0 ± 1.5[i]
Peroxide value[e]	2.8 ± 0.1[g]	0.5 ± 0.1[h]	0.4 ± 0.0[h]
FOS[f]	0.00 ± 0.00[g]	3.63 ± 0.07[h]	5.89 ± 0.06[i]
% Polymer	1.02 ± 0.01[g]	8.55 ± 0.10[h]	26.28 ± 0.75[i]

[a]Average of three replicates ± standard deviation.
[b]FFA, free fatty acid (as % linoleic acid).
[c]Unit of measure = mg KOH/ g sample.
[d]Unit of measure = absorbance/g sample, measures the amount of aldehydes, principally, 2-alkenals and 2,4-dienals.
[e]Unit of measure = milliequivalents peroxide/1000 g sample.
[f]FOS = food oil sensor. Values with the same letter (g,h,i) are not significantly different from each other at the 5% level for the same analysis. See Table 1 for company source.

TABLE 4
Oil Analyses[a] of Trilinolein Heated at 190°C (12 h/d)

Oil analysis	Day 0 (0 h)	Day 1 (12 h)	Day 2 (24 h)
Free fatty acid value[b]	0.08 ± 0.00[g]	0.24 ± 0.00[h]	0.39 ± 0.05[i]
Acid value[c]	0.12 ± 0.00[g]	0.27 ± 0.07[h]	0.82 ± 0.01[i]
p-Anisidine value[d]	3.6 ± 0.1[g]	291.4 ± 4.4[h]	335.1 ± 2.9[i]
Peroxide value[e]	5.7 ± 0.9[g]	2.1 ± 0.2[h]	0.6 ± 0.2[i]
FOS[f]	0.00 ± 0.00[g]	2.77 ± 0.10[h]	5.13 ± 0.03[i]
% Polymer	0.31 ± 0.06[g]	9.67 ± 2.27[h]	21.12 ± 0.45[i]

[a]Average of three replicates ± standard deviation.
[b]FFA, free fatty acid (as % linoleic acid).
[c]Unit of measure = mg KOH/ g sample.
[d]Unit of measure = absorbance/g sample, measures the amount of aldehydes, principally, 2-alkenals and 2,4-dienals.
[e]Unit of measure = milliequivalents peroxide/1000 g sample.
[f]FOS = food oil sensor. Values with the same letter (g,h,i) are not significantly different from each other at the 5% level for the same analysis.

Research Corporation, Monterey Park, CA) with software from Lee Scientific, Inc. The chromatographic data were collected and analyzed with a Hyundai 386SX PC and Baseline software (Waters Chromatography, Milford, MA) (10).

Data analysis. Quantitative SFC analysis of each oil sample was based upon an internal standard and a response factor. Analyses were conducted in triplicate. Standard deviations were determined for each set of replicates. Component concentrations were calculated from the following equation: $C_c = A_c(C_i/A_i)$, where C_c is the oil component concentration, A_c is the peak area for the oil component, C_i is the internal standard concentration, and A_i is the peak area for the internal standard. The percentage recovery was calculated by dividing the concentration of EPG-08 linoleate and trilinolein, obtained from the SFC results, by the concentration of sample as prepared, and then dividing the result by the percentage recovery for the unheated sample.

The reaction rate constant, k, was an "average" apparent reaction rate constant, where:

$$\ln([S]/[S_o]) = k_{\mathrm{AVE}}t \quad [1]$$

$$k_{\mathrm{AVE}}t = k_{1(T1)}\cdot t^1 + k_{2(T2)}\cdot t^2 + k_{3(T3)}\cdot t^3 + k_{4(\mathrm{T4})}\cdot t^4, \text{ etc.} \quad [2]$$

The substrate concentration (the unmodified or unoxidized TAG components in EPG-08 oleate, EPG-08 linoleate, triolein or trilinolein) at time zero is $[S_o]$, and the substrate concentration at any other time is $[S]$. The k at $T1$ is $k_{1(T1)}$, at $T2$ the k is $k_{2(T2)}$, etc., where $T1$, $T2$, $T3$, $T4$, etc. are the oil temperatures during each 24-h heating period, which includes the heating temperature (190°C), the ambient temperature after cooling (22°C), and the temperatures between 22 and 190°C during the cooling and heating periods. The time intervals t^1, t^2, t^3, t^4, etc. correspond to the time intervals when the oil was at $T1$, $T2$, $T3$, $T4$, etc., respectively (10). Linear regression analysis (12) was used to determine the "average" apparent k for each oil.

RESULTS AND DISCUSSION

HPSEC was used to determine when each of the oil samples had reached the target level of polymerization (≥20%) (Tables 1–4). Previous work indicated that the concentration of polar compounds [27–28% polar material, as determined by column chromatography (CC)] could be used as an indication that an oil is considered excessively deteriorated and should be discarded (13–15). However, others (16) have suggested that a value of 27% for the polar material may not be the best objective criterion for discarding oil, because it is based on a nonlinear correlation of oxidized fatty acids. With the advancement in column performance (reduction in particle size and increased rigidity) for HPSEC, HPSEC is considered a much better method of analysis (8,9) [much faster (30 min) and more reproducible] for heated oils than CC. In addition, the high-molecular-weight (HMW) compounds, as determined by HPSEC, may serve as a more useful indicator of oil deterioration because of their low volatility and increased stability (9). Others have used HPSEC successfully to analyze heated oils, especially the HMW compounds formed during heating (8,9).

Some investigators have correlated the concentration of polar compounds to the polymeric material content (8) or to the percentage of oxidized FFA (8,13). Oil analyses, such as FFA content, AV, *p*-AV, peroxide value (PV), iodine value (IV), and viscosity, have been used to estimate oil degradation in heated oils. None is considered completely satisfactory because each measures a limited number of oxidation products (9,17). In addition to an endpoint determination for heated frying oils based on the percentage polar compounds, an endpoint can be determined from HPSEC analysis. Previous research has indicated that an oil was no longer considered acceptable for frying if it contained ≥20% polymeric material (8). Billek *et al.* (13) reported that the percentage polymeric material could be indirectly correlated to the percentage polar material by correlation with the percentage oxidized fatty acids. In that report, 15% polymeric material in the oil corresponded to 27% polar material and indirectly corresponded to 1% oxidized fatty acids. One percent of oxidized fatty acids (petroleum ether-insoluble) is considered one of three separate parameters that could be used for determining the discard point, as proposed by the German Society for Fat Research. There seems to be sufficient justification for selecting a discard endpoint when the oil contains anywhere from 15 to 20% polymeric material, so an endpoint of 20% was selected. EPG-08 linoleate contained ≥20% polymer (defined as percentage dimer + percentage higher MW polymers) at the end of 24 h of heating (day 2) (Table 3). Figure 1 is the HPSEC separation of EPG-08 linoleate after 0, 12, and 24 h of heating at approximately 190°C. Based on an equation de-

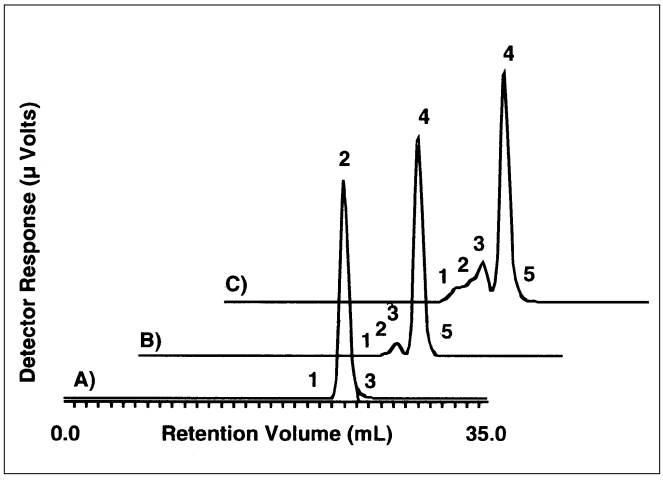

FIG. 1. High-performance size-exclusion chromatography of heated (190°C, 12 h/d) EPG-08 linoleate (ARCO Chemical Co., Newtown Square, PA). A) Day 0 (as received) peak 1 = dimeric triacylglycerol (TAG), peak 2 = monomeric TAG, and peak 3 = low-molecular-weight (LMW) products; B) Day 1 (12 h) peak 1 = tetrameric TAG, peak 2 = trimeric TAG, peak 3 = dimeric TAG, peak 4 = monomeric TAG, and peak 5 = LMW products; C) Day 2 (24 h) peak 1 = tetrameric TAG, peak 2 = trimeric TAG, peak 3 = dimeric TAG, peak 4 = monomeric TAG, and peak 5 = LMW products.

rived from the curvilinear relationship between percentage polymer and heating time, EPG-08 linoleate reached 20% polymer formation after approximately 20.5 h of heating, while trilinolein reached 20% polymer formation after approximately 22.8 h. Figure 2 is the HPSEC separation of trilinolein after 0, 12, and 24 h of heating. It has been suggested that "diene rich" oils have discard times of approximately 24 h (8). Because trilinolein and EPG-08 linoleate are highly enriched in terms of diene fatty acid content, these times are comparable.

The EPG-08 oleate sample contained 20% polymer after an estimated 33.0 h of heating, whereas the triolein sample contained 20% polymer after an estimated 51.1 h of heating. The "monene-rich" oils should have discard times of 32 h or greater (8), which was the result for EPG-08 oleate and triolein.

Generally, there was a statistically significant ($P < 0.05$) increase in the FFA value, AV, *p*-AV, FOS readings and the percentage polymer content after each 12-h heating period for each oil (Tables 1–4). The PV generally decreased after each heating period, as expected. The aldehyde (*p*-AV) content increased substantially after heating on day 1 (12 h) for the linoleic acid-containing oils. The reduction in linoleic acid content in each oil after 12 h of heating may have reduced the rate of aldehyde formation. Some investigators have indicated that the FFA content does not correlate particularly well with the polymeric material content (18). The correlation between FFA content and polymer content may differ among different fatty acids.

Peroxides were present at reduced concentrations after the first day of heating (Tables 1–4), as expected owing to the rapid decomposition of peroxides that occurs at temperatures greater than 100°C (9,19). PV determination is best used as an indicator of oxidation at storage temperatures well below 100°C (9). The low values suggest that there was little if any oxidation during the overnight refrigerated storage of the oil samples.

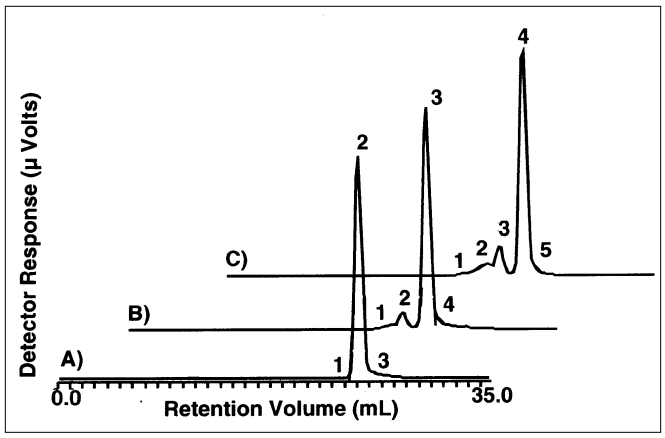

FIG. 2. High-performance size-exclusion chromatography of heated (190°C, 12 h/d) trilinolein. A) Day 0 (as received) peak 1 = dimeric triacylglycerol (TAG), peak 2 = monomeric TAG, and peak 3 = low-molecular-weight (LMW) products; B) Day 1 (12 h) peak 1 = trimeric TAG, peak 2 = dimeric TAG, peak 3 = monomeric TAG, and peak 4 = LMW products; C) Day 2 (24 h) peak 1 = tetrameric TAG, peak 2 = trimeric TAG, peak 3 = dimeric TAG, peak 4 = monomeric TAG, and peak 5 = LMW products.

FOS readings increased with heating time (Table 1–4), indicating an increase in the dielectric constant or the ratio of polar to nonpolar material. The increase in FOS values is probably due to an increase in the polarity of the nonvolatile oxidation products retained in the oil, as well as an increase in the total amount of LMW products. After 24 h of heating, a total EPG-08 linoleate polymer concentration of 26.3% corresponded to a reading of 5.89 on FOS. The polymer/FOS ratio increased from 2.4 for day 1 to 4.5 for day 2, suggesting that the rate of polymer formation exceeded the rate of polar compound formation, or more likely, the rate of polar volatile compound retention in the oil. Similarly, the polymer/FOS ratio for trilinolein increased from 3.5 to 4.1 from day 1 to day 3. The polymer/FOS ratio for triolein increased from 1.1 for day 1 to 2.2 for day 3, and the polymer/FOS ratio for EPG-08 oleate increased from 1.6 (day 1) to 2.6 (day 3).

SFC provides much greater specificity than does HPSEC for direct determination of TAG concentration. In contrast to HPSEC, TAG differing by a single double bond (e.g., OLL and LLL where O is oleic acid and L is linoleic acid) can be separated with capillary SFC (20), given that the appropriate stationary phase is used (21). Therefore, any change in the TAG structure, induced as a result of oxidation (cleavage of a 5–6 carbon fraction, formation of a hydroxy fatty acid from the addition of a hydroxy radical and an alkyl radical, or even crosslinking *via* a carbon-carbon bond between two adjacent FA on the same TAG), would result in a noticeable change in the retention time with capillary SFC, in contrast to HPSEC. There are HPLC techniques available for the separation of mixtures of TAG based on fatty acid composition. However, they have not been applied to the analysis of heated fats and oils (22).

Summaries of the substrate concentrations for each oil sample, as well as the substrate recoveries and losses during heating (SFC), are presented in Tables 5–8. The percentage of each TAG present at day 0 was equivalent to a recovery of 100%, and the values for the percentage TAG remaining in the sample for each day of heating were divided by the values for day 0 to obtain the percentage recovery. Overlay chromatograms of day 0 and day 2 for EPG-08 linoleate (Fig. 3) and trilinolein (Fig. 4) are shown and it is apparent that there were substantial substrate losses as the result of heating. The components of EPG-08 linoleate that eluted from 47.5 to 80 min have been tentatively identified as EPG-08 linoleate TAG, whereas the components eluting between 35 min and 47 min were tentatively identified as EPG-08 linoleate diacylglycerols. The TAG components eluting from 47.5 to 80 min represent the range in mole ratio of propylene oxide to glycerol obtained from an initial reaction mixture of 8 moles of propylene oxide to one mole of glycerol. Each additional peak from 47.5 to 80 min represents an increase in one of the ratios of propylene oxide to glycerol, which indicates that

TABLE 5
EPG-08 Oleate Losses During Heating

Time	Sample conc.[a] (mg/mL)	EPG-08 TG conc. (mg/mL)	%EPG-08 TG[b] in sample	Recovery (%)
Day 0	25.1	22.1[c] ± 0.1[d]	88.0	100.0
Day 1	25.3	15.9 ± 0.1	63.1	71.7
Day 2	25.0	11.4 ± 0.1	45.8	52.0
Day 3	25.2	8.9 ± 0.4	35.5	40.4

[a]Concentration of sample as weighed.
[b]For Day 0, 88.0% of the EPG-08 sample is triacylglycerol (TG), while the remainder (12.0%) is diacylglycerol (DAG). After Day 0, the remainder is DAG and a variety of oxidized species. See Table 1 for company source.
[c]Concentration of sample calculated from supercritical fluid chromatography (SFC) data and corrected for the response factor. The response factor is equal to the total sample (as weighed)/total sample (as determined by SFC).
[d]Standard deviation for three replicates.

TABLE 6
Triolein Losses During Heating

Time	Sample conc.[a] (mg/mL)	Triolein conc.[b] (mg/mL)	Recovery (%)
Day 0	0.469	0.470 ± 0.012[c]	100.0
Day 1	0.410	0.282 ± 0.003	68.6
Day 2	0.451	0.219 ± 0.015	48.7
Day 3	0.444	0.171 ± 0.006	38.5
Day 4	0.482	0.159 ± 0.009	33.0
Day 5	0.456	0.087 ± 0.002	19.0

[a]Concentration of sample as weighed.
[b]Concentration of sample calculated from SFC data and corrected for response factor. The response factor is equal to the total sample (as weighed)/total sample (as determined by SFC). See Table 5 for other abbreviation.
[c]Standard deviation for three replicates.

TABLE 7
EPG-08 Linoleate Losses During Heating

Time	Sample conc.[a] (mg/mL)	EPG-08 TG conc. (mg/mL)	%EPG-08 TG[b] in sample	Recovery (%)
Day 0	24.9	22.8[c] ± 0.2[d]	91.3	100.0
Day 1	25.1	17.9 ± 0.4	70.8	77.5
Day 2	25.2	12.5 ± 1.6	49.6	54.3

[a]Concentration of sample as weighed.
[b]For Day 0, 91.3% of the EPG-08 sample is triacylglycerol (TG), while the remainder (8.7%) is diacylglycerol (DAG). After Day 0, the remainder is DAG and a variety of oxidized species. See Table 1 for company source.
[c]Concentration of sample calculated from SFC data and corrected for the response factor. The response factor is equal to the total sample (as weighed)/total sample (as determined by SFC). See Table 5 for other abbreviation.
[d]Standard deviation for three replicates.

TABLE 8
Trilinolein Losses During Heating

Time	Sample conc.[a] (mg/mL)	Trilinolein conc.[b] (mg/mL)	Recovery (%)
Day 0	0.419	0.419 ± 0.040[c]	100.0
Day 1	0.403	0.238 ± 0.005	59.0
Day 2	0.432	0.181 ± 0.002	41.8

[a]Concentration of sample as weighed.
[b]Concentration of sample calculated from SFC data and corrected for the response factor. The response factor is equal to the total sample (as weighted)/total sample (as determined by SFC). See Table 5 for abbreviation.
[c]Standard deviation for three replicates.

there are 14 separate mole ratio products formed in significant amounts during the propoxylation step.

A plot of $\ln([S]/[S_o])$ vs. time for the loss of unmodified TAG substrate during heating indicated that the reaction for the loss of EPG-08 linoleate and trilinolein after heating was an apparent first-order reaction. The average apparent first-order reaction rate constant (as determined by SFC) for EPG-08 linoleate was $0.0253 \pm 0.0032\ h^{-1}$, while the rate for trilinolein was $0.0348 \pm 0.0034\ h^{-1}$, which indicated that the degradation rate for trilinolein was significantly greater than the rate for EPG-08 linoleate. Part of the difference between the k for the two oils may have been due to small differences in the frying oil temperature. It is difficult to maintain the oil temperature within a narrow range in a small fryer. The correlation coefficient was 0.91 for the plot of $\ln([S]/[S_o])$ vs. time for EPG-08 linoleate, while the correlation coefficient was 0.94 for the plot of $\ln([S]/[S_o])$ vs. time for trilinolein. Linear regression analysis (12,23) indicated that the rate for the trilinolein substrate loss was significantly greater than the rate for the EPG-08 linoleate loss ($P < 0.05$). In contrast, a comparison of the k for EPG-08 oleate, which was $0.0252 \pm 0.0008\ h^{-1}$, with the rate for triolein, which was $0.0256 \pm 0.0011\ h^{-1}$, indicated that the two degradation rates were not significantly different.

Theoretically, determination of the rate of degradation, based on an accurate analysis of the substrate concentration, should be more accurate than a rate determined from a concentration determination for one or several reaction products, particularly because a particular reaction product concentration could be altered by slight differences in heating conditions, reaction chemistry, or substrate structure. The HPSEC data (percentage polymer) do not agree with losses predicted from the SFC data. The SFC results indicate that less than 40% of the original trilinolein substrate remained at the end of the 24-h heating treatment, while the HPSEC results suggest that over 78% of the original trilinolein substrate remains after heating. Similar large differences exist between the SFC and HPSEC data for the EPG-08 linoleate concentration remaining after heating. The results suggest that, although HPSEC analysis may be an excellent analysis for monitoring frying oil quality, it may not accurately reflect the loss of TAG substrate during heating, and that one needs to be careful about drawing inferences about the rate of oxidation or oil stability based on tests specifically designed for a limited number of oxidation products. The HPSEC data suggest that much of the material that co-elutes with the "monomer" component during HPSEC has been altered, and further evaluation of this material is warranted.

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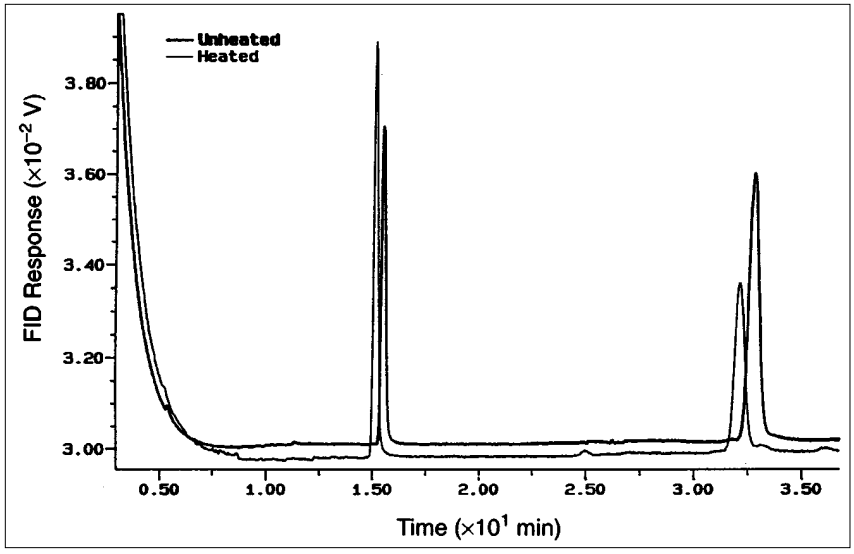

FIG. 3. The capillary supercritical fluid chromatogram of day 0 and day 2 (24 h) for EPG-08 linoleate. The upper chromatogram is day 2 (thick line, heated), while the lower chromatogram (thin line, unheated) is day 0. The internal standard (tridecanoin) eluted at approximately 21 min. The diacylglycerol EPG-08 linoleate components eluted from 35–47.5 min, while the triacylglycerol EPG-08 linoleate components eluted from 47.5–78 min; FID, flame-ionization detector. See Figure 1 for company source.

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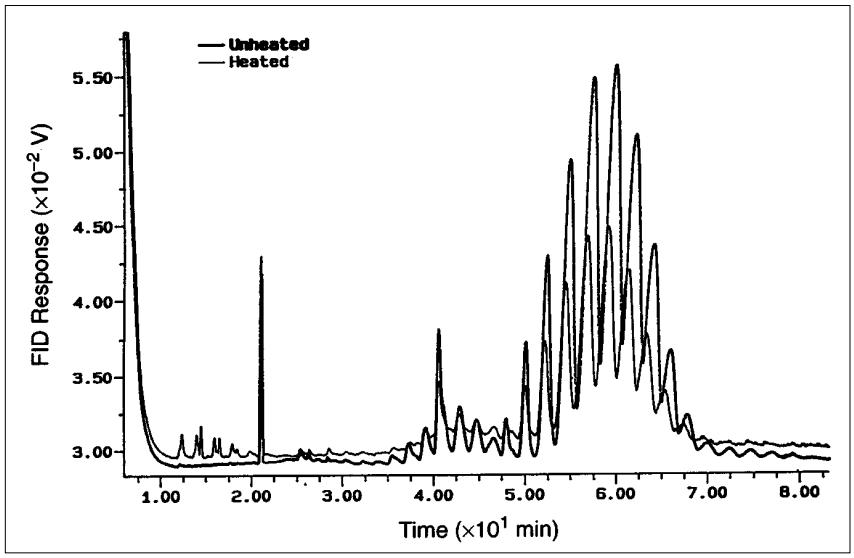

FIG. 4. The capillary supercritical fluid chromatogram of day 0 and day 2 (24 h) for trilinolein. The upper chromatogram is day 2 (thick line, heated) and the lower is day 0 (thin line, unheated). The internal standard (tridecanoin) eluted at approximately 15 min, and the trilinolein eluted at 32–33 min. See Figure 3 for abbreviation.

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