Nonvolatile Components Produced in Triolein During Deep-Fat Frying

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Triolein was heated at 190°C (375°F) in a deep-fat fryer for 12 h/day until high-performance size-exclusion chromatography indicated polymer formation had exceeded 20%. Increases in the free fatty acid, total acid value, food oil sensor and *p*-anisidine values upon heating indicated that thermal oxidation and degradation of triolein had occurred. After the initial sample (day 0), the peroxide values decreased to very low values. The amount of polymeric triacylglycerol material increased during heating. Linear regression analysis of percent polymer vs. heating time indicated that the sample would contain ≥20% polymers after 51.1 h of heating. Capillary supercritical fluid chromatography (SFC) was used to determine the percentage of triolein remaining after 12, 24, 36, 48 and 60 h of heating, which was 68.6, 53.9, 35.9, 33.0 and 19.0%, respectively. The average reaction rate constant (apparent first-order) for the change in triolein concentration, SFC, during heating was 0.0256 ± 0.0011 h^{-1}.

KEY WORDS: High-performance size-exclusion chromatography, nonvolatile oxidation products, supercritical fluid chromatography, triolein, trioleylglycerol.

Oxidation and heating studies have been conducted on numerous lipid substrates, including methyl oleate and linoleate (1), trilinolein, triolein and tristearin (2), ethyl eicosapentaenoate, docosahexaenoate, linoleate and linolenate (3), trilinoleylglycerol (4), trilinolenylglycerol (5) and synthetic triacylglycerols (TAGs) containing linoleate and linolenate (6) and mixtures of unsaturated and saturated TAGs (7). Classical oxidation analyses and high-performance size-exclusion chromatography (HPSEC) (7,8) have been used to monitor secondary oxidation products in heated fats and oils, such as free fatty acids (FFAs), total acids, 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. For example, an assay that has little utility for monitoring oil degradation during deep-fat frying is the peroxide value (PV) analysis. To determine oil stability, quantitation of either all of the oxidation products or the substrate would be the most accurate method. Capillary supercritical fluid chromatography (SFC) can be used to directly quantitate individual TAG substrate components and should be more accurate than any technique that measures a single oxidation product or even a group of oxidation products.

Oils used in deep-fat frying typically contain >96% TAG (monomer), <4% polar material, 0.5% polymeric material, 0.02% FFAs, 0.01% oxidized FFA and 0–7 ppm soaps prior to heating (9). As the oil is heated, the TAG concentration decreases, and the concentration of polar compounds, polymeric material, FFA, oxidized FFA and soaps increase (9).

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One area of concern is the type of polymerization products that may form when oils are heated (10). Earlier research on frying oils indicated that consumption of oil with excessive cyclic compounds (11), excessive thermal abuse (12) or an excessive amount of lower-molecular weight (LMW) compounds may affect health deleteriously (10). It has been suggested that TAG dimers and oxidized monomers are the problem compounds, not the larger polymers (13). The larger polymeric material is not absorbed as well as the dimeric and monomeric material (10). It is thought that most of the early feeding study samples had much greater dimer and oxidized monomer concentrations than humans would normally ingest. Kubow (10) provided extensive details about the toxic consequences. Toxicity may not be a major problem in food oils, although further testing is needed (10).

Nonvolatile analyses were done on triolein to determine the amount and type of decomposition products formed during heating. The objective was to monitor the change in triolein that occurred during deep-fat frying.

EXPERIMENTAL PROCEDURES

Oil sample preparation. Approximately 3.8 L of triolein, synthesized from oleic acid [≥99% by gas chromatography (GC)] and glycerol (ARCO Chemical Co., Newtown Square, PA), without added antioxidants, was heated in a deep-fat fryer (Model F175A; Intedge Industries, Inc., Whippany, NJ) at 190°C for 12 h per day until polymer concentration exceeded 20%. The capacity of the fryer was 5.58 kg, and the surface area of the oil was 610 cm^2 (20.2 cm × 30.2 cm). The fryer was approximately one-third full of oil. After each 12-h heating period, the fryer was turned off, and the oil was allowed to cool to approximately 90–95°C. Then, an oil sample of approximately 100–150 mL was collected in an amber glass bottle, blanketed with nitrogen, capped, sealed with parafilm, and stored in a refrigerator (approximately 2–5°C) until further analysis. The remaining oil and fryer were covered with aluminum foil until the next 12-h heating period. Oil analyses were conducted the next morning or as soon as possible thereafer.

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 mm × 7.8 mm) (Phenomenex, Torrance, CA) followed by two Phenogel (5 μ, 500 Å and 100 Å) columns (500 mm × 8.0 mm) in series connected to a evaporative light-scattering detector (ELSD IIA; Varex Corp., Burtonsville, MD).

Tetrahydrofuran (THF) was the mobile phase (1.0 mL/min). THF (Optima; Fisher Scientific, Fair Lawn, NJ) was filtered with 0.45 μ HV discs (Millipore Corp., Bedford, MA), and degassed before use. THF was kept under constant nitrogen gas purge while in use, and no butylated hydroxytoluene (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. Ultra-high purity (99.999%) nitrogen gas was used.

Polypropylene glycol (PPG) MW standards of 4000, 3000, 2000, 1000 and 425 (Aldrich Chemical Co., Milwaukee, WI) were used to estimate sample component MWs as a function of retention volume (V_r). PPG MW standards were prepared at approximately 20 mg/mL in THF, filtered with 0.45 μ HV discs, and stored in an amber vial in a refrigerator until use. The log of the MW of PPG standards was plotted vs. V_r. The MWs of the heated oil components were estimated from their V_r values from Equation 1.

$$\log MW = 6.2671 + (-0.13564)(V_r) \quad [1]$$

Peak area was determined with the Dynamax Method Manager Software Version 1.3.1 (Rainin Instrument Co.).

Oil samples were prepared for HPSEC analysis by dissolving a 30-μL sample in 2970 μL THF (1:100) and then by filtering with a 0.45 μHV disc. All analyses were conducted in triplicate, and standard deviations were determined.

FFA value. FFA values (as % oleic acid) of oil samples were determined by Official Method Ca 5a-40 of the American Oil Chemists' Society (AOCS) (14).

Acid value (AV). AVs (mg KOH/g sample) of oil samples were determined by AOCS Official Method Cd 3d-63 (14).

p-Anisidine value (p-AV). The *p*-AV's of oil samples were determined by AOCS Official Method Cd 18-90 (14).

PV. PVs (milliequivalents peroxide/1000 g sample) of oil samples were determined by AOCS Official Method Cd 8b-90 (14).

Food oil sensor (FOS). The FOS (Model No. NI-21A; Northern Instrument Co., Lino Lakes, MN) measures the dielectric constant of oil. Unheated triolein was used to calibrate the FOS and determine the zero point. The procedure followed was outlined in the manual.

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

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 samples were accurately weighed to ±0.00001 g and prepared at a concentration of approximately 2 mg/mL, and the internal standard concentration was approximately 1 mg/mL. Prior to injection, samples were diluted to approximately 0.4 mg/mL, and the internal standard was diluted to approximately 0.2 mg/mL.

Apparatus. The supercritical-fluid chromatograph was a Lee Scientific Model β 501 (Lee Scientific, Inc., Div. Dionex, Salt Lake City, UT) with a Valco A90 injector (Houston, TX) with a 0.2-μL internal loop operated in a timed-split mode with an injection time of 0.1 s. The capillary column 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 with 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 flame-ionization detection temperature was 375°C. The oven, pump and injector were controlled by an ARC Turbo PC (American 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).

Data analysis. Quantitative analysis of triolein for SFC was determined based upon the internal standard and the response factor. Analyses were conducted in triplicate. Standard deviations were determined for each set of replicates.

Linear regression (15) analysis was used to compare the HPSEC and the SFC results. Analysis of variance with LSD was used for the statistical analysis of the results from the lipid oxidation analyses.

The reaction rate constant, k, has been defined as an "average" apparent reaction rate constant, where $ln(S/S_0) = kt$ and $kt = k_{1(T_1)}*t^1 + k_{2(T_2)}*t^2 + k_{3(T_3)}*t^3 + k_{4(T_4)}*t^4$, etc. The substrate or triolein concentration at time zero is

TABLE 1

Analysis of Triolein Heated at 190°C (12 h/day)

Time (h)	FFA[a]	Acid value[c]	*p*-Ansidine value[d]	Peroxide value[e]	FOS[f] reading
0	0.1 ± 0.0[b,g]	0.1 ± 0.0[g]	2.1 ± 0.4[g]	2.9 ± 0.1[g]	—[g]
12	0.3 ± 0.0[h]	0.5 ± 0.1[h]	48.3 ± 0.3[h]	1.3 ± 0.1[h]	2.16 ± 0.01[h]
24	0.4 ± 0.1[h,i]	0.7 ± 0.1[i]	72.8 ± 0.4[i]	0.3 ± 0.1[i]	3.61 ± 0.02[i]
36	0.4 ± 0.0[j]	0.9 ± 0.0[j]	87.9 ± 1.5[j]	0.2 ± 0.1[i,j]	5.22 ± 0.03[k]
48	0.4 ± 0.0[j]	1.1 ± 0.1[k]	97.9 ± 2.4[k]	0.1 ± 0.0[j,k]	6.72 ± 0.03[k]
60	0.6 ± 0.1[k]	1.4 ± 0.0[l]	98.3 ± 2.2[k]	0.0 ± 0.0[k]	8.45 ± 0.03[l]

[a]FFA = free fatty acid (as % oleic acid).
[b]Average of three replicates ± standard deviation.
[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.
[g,h,i,j,k,l]Values with the same letter are not significantly different from each other at the 5.0% level.

S_0, and the substrate concentration at any other time is S. The k at T_1 is $k_{1(T_1)}$, at T_2 the k is $k_{2(T_2)}$, etc., where T_1, T_2, T_3, T_4, 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 T_1, T_2, T_3, T_4, etc., respectively.

RESULTS AND DISCUSSION

HPSEC was used to determine when the triolein sample had reached a target level of polymerization (20%). Previous work indicated that the concentration of polar compounds [determined by column chromatography (CC)] or oxidized FFA could be used to determine when an oil is considered excessively deteriorated and should be discarded (16). However, others (17) feel 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 FA. With the advancement in column performance (reduction in particle size and increased rigidity), HPSEC is considered a much more efficient method (7,8) [much faster (30 min) and more reproducible] of analysis for heated oils. 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 (8). The major drawback to HPSEC is the equipment cost relative to CC. Others have used HPSEC successfully to analyze heated oils, especially the HMW compounds formed during heating (7,8). Some investigators have correlated the amount of polar compounds to polymeric material (7) or to oxidized FFA and polymeric material (7,16). Oil analyses such as FFA, AV, *p*-AV, PV, iodine value (IV) and viscosity have been used to estimate oil degradation in heated oils. None are considered completely satisfactory (8,18).

The MW of the components in each peak was determined by Equation 1 (in the Experimental Procedures section) for HPSEC. Previous work indicated that oil was no longer acceptable for frying if it contained ≥20% polymeric material (7). Triolein reached ≥20% polymer formation (defined as dimer, trimer and tetramer) on day 5 (60 h) (Table 2). Figure 1 shows the HPSEC separation after 0 and 60 h of heating at 190°C. Linear regression indicated that triolein reached 20% polymer formation after 51.1 h of heating at 190°C. Others (7) have indicated that monoene-rich oils that have been air-heated have discard times of 32 h. Molecular weight distributions (MWD), as determined by HPSEC V_r values, indicated higher MW

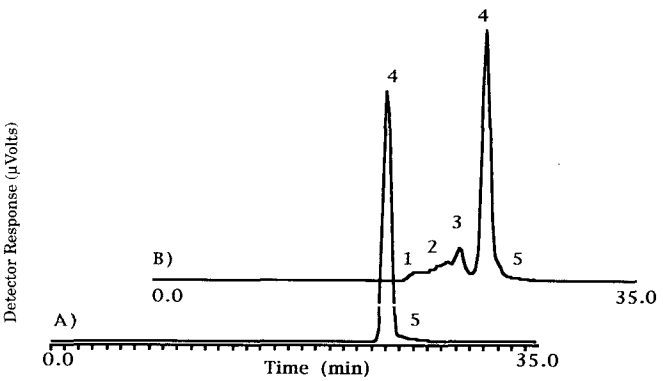

FIG. 1. (A) High-performance size-exclusion chromatogram (HPSEC) of Day 0 (nonheated, 0 h) triolein: peak 4 = triacylglycerol (TAG) monomer and peak 5 = LWM (low-molecular weight) products. (B) HPSEC of Day 5 (60 h) triolein: peak 1 = TAG tetramer, peak 2 = TAG trimer, peak 3 = TAG dimer, peak 4 = TAG monomer, and peak 5 = LMW products.

TABLE 2

Molecular Weight Distribution of Triolein Heated at 190°C (12 h/day) as Determined by High-Performance Size-Exclusion Chromatography

	Time (h)					
Sample	0	12	24	36	48	60
Tetramer[a]						
V_r (mL)	—	—	—	19.2 ± 0.0[b]	18.9 ± 0.1	19.0 ± 0.0
% Area	—	—	—	0.4 ± 0.0	1.0 ± 0.2	4.2 ± 0.2
MW[c]	—	—	—	4600	5100	4900
Trimer						
V_r (mL)	—	21.0 ± 0.0	21.0 ± 0.0	21.0 ± 0.0	21.0 ± 0.0	21.0 ± 0.0
% Area	—	0.4 ± 0.0	2.0 ± 0.1	4.0 ± 0.1	7.3 ± 0.0	11.2 ± 0.2
MW	—	2600	2600	2600	2600	2600
Dimer						
V_r (mL)	—	22.3 ± 0.0	22.3 ± 0.0	22.3 ± 0.0	22.3 ± 0.0	22.3 ± 0.0
% Area	—	1.9 ± 0.1	4.6 ± 0.1	7.0 ± 0.2	9.1 ± 0.1	11.1 ± 0.2
MW	—	1700	1700	1700	1700	1700
Monomer						
V_r (mL)	24.2 ± 0.0	24.2 ± 0.2	24.2 ± 0.0	24.2 ± 0.0	24.2 ± 0.0	24.2 ± 0.0
% Area	92.3 ± 0.1	90.7 ± 0.5	87.4 ± 0.6	83.7 ± 0.5	77.9 ± 0.1	69.8 ± 0.2
MMW	970	960	970	970	970	970
LMW Prod.						
V_r (mL)	32.2 ± 0.4	32.1 ± 0.7	31.0 ± 0.5	30.6 ± 0.2	30.4 ± 1.1	29.5 ± 0.3
% Area	7.7 ± 0.1	7.0 ± 0.5	6.0 ± 0.7	4.9 ± 0.7	4.7 ± 0.2	3.7 ± 0.2
MW	80	80	120	130	140	180

[a]Tentative identity based on molecular weight estimates.
[b]Average of three replicates ± standard deviation.
[c]Average molecular weight estimated based on retention volume (V_r).

(*ca.* 975 g/mole) for the HPSEC monomer peak components as compared to the actual MW of triolein (MW = 885.45 g/mole). The higher MW value may be due to the use of PPG MW standards, which may have different hydrodynamic volumes than TAG. Currently, there are no polymeric TAG MW standards available for calibrating HPSEC columns, a factor that may result in an overestimation of TAG MWs.

Generally, there was a statistically significant ($P < 0.05$) increase in the FFA values, the AV, the *p*-AV and FOS reading after each 12-h heating period (Table 1). The most notable exception was the absence of a statistically significant change in the FFA value from 24 to 48 h of heating. The PV generally decreased after each heating period, as expected. Triolein FFA and AV increased with increased heating (Table 1). Other investigators have found similar results for FFA values and AV for oil used for deep-fat frying (2). On Day 5 (60 h), a FFA concentration of 0.6% and an AV concentration of 1.4% corresponded to 26.5% polymer (Tables 1 and 2). The aldehyde (*p*-AV) content increased substantially after day 1 (12 h) and less so thereafter, reaching a plateau at Days 4 (48 h) and 5 (Table 1), thus possibly indicating that polymer formation exceeded aldehyde formation at the later stages of heating, or that the decreased monomer concentration resulted in reduced aldehyde production and concentration. Earlier work determined that 1.0% oxidized FA corresponded roughly to 15% polymeric material for heated TAG (16). Others have indicated that the FFA content does not correlate particularly well with the polymeric material content (19).

Peroxides were only present in low concentrations after the first days of heating (Table 1) due to the rapid decomposition of peroxides at temperatures above 100°C (8,20). PV determination is a good indicator of oxidation only at lower temperatures (8). Low values suggest that there was little oxidation during overnight refrigerated storage of the sample.

FOS readings increased with heating time (Table 1), indicating an increase in the dielectric constant or the ratio of polar to nonpolar components. The increase in FOS values may be due to an increase in the polarity of the oxidation products retained in the oil and not the total amount of LMW products because the LMW product concentration decreased during heating (Table 2). After 60 h of heating, a total polymer concentration of 26.5% corresponded to an FOS reading of 8.45. The polymer/FOS ratio increased from 1.06 for Day 1 (12 h) to 3.13 for Day 5 (60 h), suggesting that the rate of polymer formation exceeded the rate of polar compound formation.

SFC provides much greater specificity than does HPSEC for determining substrate 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 (21) with an appropriate stationary phase (22). Therefore, any change in the TAG structure induced as a result of oxidation (cleavage of a 5-6 carbon fraction, formation of a hydroxy FA from the addition of a hydroxy radical and an alkly radical, or even crosslinking *via* a carbon-carbon bond of two adjacent FAs on the same TAG) would result in a substantial change in the retention time with capillary SFC, unlike with HPSEC.

Summaries of the concentrations of the samples, as well as the recoveries and losses of triolein during heating (SFC and SEC), are presented in Table 3. The percentage of triolein from Day 0 was equivalent to a recovery of 100%, and the values for the percent triolein remaining in the sample for each day of heating were divided by Day 0 to obtain the percent recovery for triolein. The chromatograms of Day 2 (24 h) and Day 5 (60 h) are presented in Figure 2.

A plot of $ln\ (S/S_0)$ vs. time for the loss of triolein during heating indicated that the reaction rate was an apparent first-order reaction for SFC data (Fig. 3), where S = substrate concentration at time t and S_0 = initial substrate concentration. The k is an "average" k because the heating was not continuous, but intermittent (12 h heating, then 12 h cooling). The average k for triolein loss during heating, SFC, was $(25.6 \pm 1.1) \times 10^{-3}\ h^{-1}$ and the correlation coefficient = 0.97, while the rate constant based on the HPSEC results was $(49.9 \pm 3.2) \times 10^{-4}\ h^{-1}$ and the correlation coefficient was 0.94. Linear regression analysis (15,23) indicated that the slope for the SFC data was significantly greater than the slope for the HPSEC data ($P < 0.05$). The $ln\ S/S_0$ data for SFC and HPSEC was not significantly different for Day 0, but was for Days 1–5 (Fig. 3). The degradation rate determined from the SFC data was over five times that predicted from the HPSEC data, which indicates that the monomer peak

TABLE 3

Triolein Recovery During Heating Supercritical Fluid Chromatography (SFC)

Time (h)	Sample conc.[a] (mg/mL)	Triolein conc.[b] (mg/mL)	% Triolein recovery
0	0.469	0.470 ± 0.012[c]	100.0
12	0.410	0.282 ± 0.003	68.6
24	0.451	0.219 ± 0.015	48.7
36	0.444	0.171 ± 0.006	38.5
48	0.482	0.159 ± 0.009	33.0
60	0.456	0.087 ± 0.002	19.0

[a]Concentration of sample as weighed.
[b]Concentration of triolein in sample calculated from SFC data and corrected for response factor.
[c]Average of three replicates ± standard deviation.

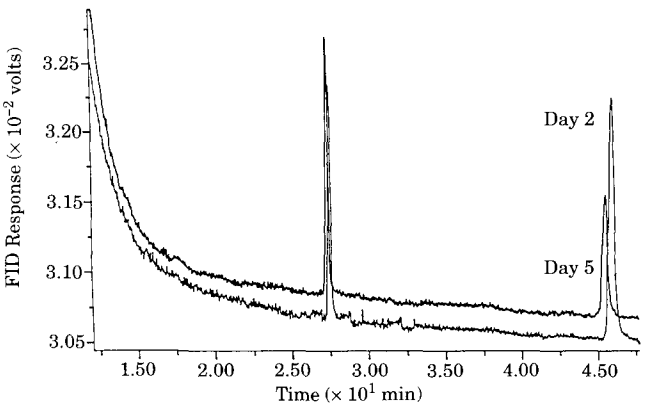

FIG. 2. Supercritical fluid chromatogram of Day 2 (24 h) and Day 5 (60 h) triolein. The upper chromatogram is Day 5 and the lower is Day 2. The internal standard (tridecanoin) eluted between 27 and 28 min, and the triolein eluted after 45 min. FID, flame-ionization detector.

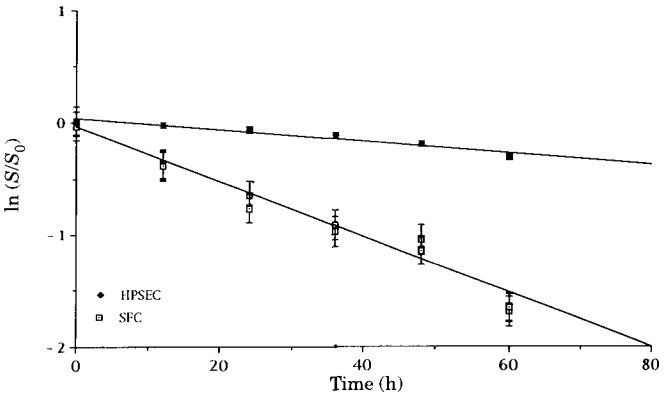

FIG. 3. Determination of "average" first-order reaction rate constants from high-performance size-exclusion (HPSEC) and supercritical fluid chromatographic (SFC) analysis of heated triolein. The substrate or triolein concentration at time zero is S_0, and the substrate concentration at any other time is S. The "average" apparent reaction rate constant ± the standard error for the HPSEC data is (49.9 ± 3.2) × 10^{-4} h^{-1}. The correlation coefficient is 0.94. For the SFC data, the correlation coefficient is 0.97 and the "average" apparent reaction rate constant is (24.5 ± 1.1) × 10^{-3} h^{-1}.

from the HPSEC data was not just trioleylglycerol but contained a substantial amount of thermally altered or oxidized substrate.

Theoretically, a determination of the rate of loss based on an accurate analysis of the substrate concentration should be more accurate than a rate determined from a concentration determination of one of 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 (% polymer) do not agree with losses predicted from the SFC data. The data 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 based on tests specifically designed for a limited number of oxidation products. HPSEC data suggest that much of the material that coelutes with the "monomer" during HPSEC has been altered, particularly toward the endpoint of 20% or more polymeric material. Further evaluation of this material is warranted.

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