Esterified Propoxylated Glycerol Soyate, a Fat Substitute Model Compound, and Soy Oil after Heating

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The changes occurring in two oil samples [EPG-00 soyate (transesterified soybean oil) and soy oil esterified propoxylated glycerol (EPG-08 soyate, a model, fat substitute compound)] were compared after heating at ~190 °C for 12 h/day. The EPG-00 soyate sample required 48 h of heating to attain a polymer content >20%, while the EPG-08 soyate required only 36 h. After 48 h of heating the EPG-00 soyate sample, the free fatty acid value (FFA) increased from 0.19 to 0.79, the acid value (AV) increased from 0.10 to 1.59, and the *p*-anisidine value (p-AV) increased from 1.6 to 195.4. In comparison, after only 36 h of heating, the EPG-08 soyate sample had FFA, AV, and p-AV increases from 0.19 to 0.71, from 0.26 to 1.36, and from 1.1 to 191.7, respectively. The triacylglycerol substrate degradation rate for EPG-00 soyate was $k = 0.0126 \pm 0.0003$ h^{-1}, while the rate for EPG-08 soyate was $k = 0.0166 \pm 0.0017$ h^{-1}. The results suggest that the EPG-00 soyate or transesterified soybean oil is slightly more stable than EPG-08 soyate.

Keywords: *Esterified propoxylated glycerol soyate; soy oil; frying oil; fat substitute*

INTRODUCTION

Several fat substitutes have been developed to mimic the unique sensory properties normally associated with fats and oils, such as flavor, mouthfeel, odor, texture, and appearance, but without the calories (Glicksman, 1991). One promising group of fat-derived fat substitutes, in addition to Olestra marketed by Proctor and Gamble (Cincinnati, OH), is the fatty acid esterified propoxylated glycerols (EPGs). These potential fat substitutes are nondigestible, nonabsorbable, low-caloric fat substitutes that have similar characteristics to vegetable oils and fats with excellent sensory properties (Cooper, 1990a,b). EPGs may be used at deep-fat frying temperatures.

Esterified epoxide-extended polyols (EEEPs) are nondigestible, nonabsorbable, low-caloric fat substitutes that may be used alone as a cooking oil or as a replacement fat in food formulation. EEEPs have similar characteristics to vegetable oils and fats and have good organoleptic properties. The magnitude of the substitution depends on the fat caloric value intended as well as the characteristic desired (White and Pollard, 1988, 1989). The EEEP fat mimetics developed by ARCO Chemical Technology, Inc. do not appreciably hydrolyze in the digestive tract and are very resistant to intestinal absorption. Studies have shown that the hydrolysis products from the EEEPs are not toxic.

Esterified propoxylated glycerins (EPGs) are fat substitutes that are a special subgroup of the general group of EEEP compounds, where glycerol is the polyol and propylene oxide is the epoxide. The general formula for the EPGs is G(PO)*n*(FE)*b*, where G is glycerol, PO is propylene oxide, FE is a fatty acyl moiety, *n* is the average propoxylation number, and *b* is the average number of fatty acids between 2 and 3 (White and Pollard, 1988). EPGs can substitute for fats and oils in products such as table spreads, frozen desserts, salad dressing, and bakery products (Anonymous, 1990). The method for the preparation of esterified propoxylated glycerin (EPG) includes preparation of propoxylated glycerol (PG), followed by transesterification and purification (Cooper, 1990a,b).

Unheated oils, prior to deep fat frying, typically contain greater than 96% triacylglycerols (monomers) and approximately 0.5% polymeric material, while the rest is comprised of polar components, free fatty acids (FFA), oxidized FFA, and soaps. During heating, the triacylglycerol (TAG) concentration decreases, and the concentration of polar compounds, polymeric material, FFA, oxidized FFA, and soap content increases substantially (Blumenthal and Stier, 1991).

Chemical changes occurring during deep fat frying are characterized by two major reactions: thermolytic and oxidative (Nawar, 1984, 1985). Thermolytic reactions occur in the absence of oxygen at elevated temperatures near 200 °C and above. The oxygen, heat, and water (from food) combine to produce decomposition products that are either volatile or nonvolatile. Polymeric material such as dimeric and trimeric TAG and lower molecular weight polar compounds, such as free fatty acids and mono- and diacylglycerols, are the nonvolatile components formed during heating. The predominant degradation products formed in polyunsaturated systems are dimeric triacylglycerols and cyclic compounds (Christopoulou, 1989a,b). The rate of lipid oxidation can also be monitored by measuring changes in the free fatty acid content, color, aldehyde concentration, and the dielectric constant (White and Wang, 1986; Yoon et al., 1987; El-Shami et al., 1992; Belbin, 1993; Warner and

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Mounts, 1993). Chemical oil analyses, such as the free fatty acid value and the *p*-anisidine value, have been used to estimate oil oxidation and deterioration during heating, none of which provide a complete representation of the chemical changes that occur (Gray, 1978; White and Wang, 1986). The physical conditions of heating such as temperature, length of heating time, oil surface area, and more importantly, the chemical and physical characteristics of the oil are important factors that determine the type of degradation products and the amount of oxidation occurring (Paquette et al., 1985). However, the complete characterization of the oxidative changes that occur in potential fat based fat substitutes upon heating, such as EPG-08 soyate, has yet to be concluded. In this study, the oxidative changes that occur in heated esterified propoxylated glycerol soyate were determined and compared to soybean oil.

MATERIALS AND METHODS

Materials. Approximately 3.4 kg of EPG-08 soyate without added antioxidants was obtained from ARCO Chemical Co. (Newtown Square, PA). EPG-08 soyate indicates a fatty acid esterified propoxylated glycerol (EPG) that was prepared with 8 mol of propylene oxide/mol of glycerol (EPG-08). Fatty acids from soybean oil were esterified to the propoxylated glycerol to form EPG-08 soyate. The EPG-08 soyate sample was heated in a deep-fat fryer (model F175A, Intedge Industries, Inc., Whippany, NJ) at ~190 ± 5 °C for 36 h (12 h/day). A second sample of approximately 3.4 kg of EPG-00 soyate was heated under the same conditions as the first sample for 48 h (12 h/day). The "00" in EPG-00 indicates that no propylene oxide was added to the glycerol, so EPG-00 soyate is essentially transesterified soybean oil.

Oil Heating Procedures. The oil temperature was monitored with a stainless steel thermocouple probe (OMEGA, Stamford, CT) connected to a 21X Micrologger (Campbell Scientific, Logan, UT). Time−temperature data were collected and averaged to improve temperature control and to follow, as close as possible, isothermal (~190 °C) conditions during heating of the oil for 12 h each day. After each heating period, the fryer was turned off, and approximately 150 mL of the sample was removed and placed in an amber bottle, blanketed with nitrogen, and stored in the dark at approximately 3−5 °C until analysis the following day. The remaining oil left in the fryer was covered with aluminum foil until the next morning for the next 12-h heating period. The unheated and heated samples were analyzed in triplicate,for their physical and chemical properties (Hansen and Artz, 1994; Hansen et al., 1994; Artz et al., 1997).

Chemical Analysis. The free fatty acid value (FFA), *p*-anisidine value (p-AV), peroxide value (PV), and acid value (AV) were determined in triplicate according to the American Oil Chemists' Society (AOCS) official methods Ca 5a-40, Cd 18−90, Cd 8b-90, and Cd 3d-63, respectively (AOCS, 1990). The data and statistical analyses were done with Microsoft Excel 4.0 (Microsoft Corp., Redmond, WA).

Physical Analysis. The color grading of samples were determined by AOCS official method Cc 13b-45 (AOCS, 1990). The Lovibond AOCS color scale (The Tintometer Co., Williamsburg, VA) consisted of Lovibond yellow scale (1.0−9.0, 10.0, 15.0, 20.0, 35.0, 50.0, and 70.0) and AOCS/Tintometer red scale (0.1−0.9, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.6, 8.0, 9.0, 10.0, 11.0, 12.0, 16.0, and 20.0). The dielectric constant was measured with the FoodOil-Sensor (Northern Instruments Corp., Lino Lakes, MN). UV absorption was measured on a Shimadzu UV-260 (Kyoto, Japan) for the wavelength range of 234 nm. Isooctane (spectrophotometric grade, Aldrich Chemical Co., Milwaukee, WI) was used as the solvent (Hansen and Artz, 1994; Artz et al., 1997).

High-Performance Size Exclusion Chromatography (HPSEC). The HPSEC system consisted of a HP drive module (Rainin Instruments Co., Woburn, MA), electronic pressure module, dual-chamber Dynamax dynamic mixer, prime purge valve, autosampler (model 728, Alcott Chromatography, Norcross, GA), a 7030 Rheodyne (Cotati, CA) switching valve, a 7125 Rheodyne injection valve with 20-μL sample loop, and a 7161 Rheodyne position sensing switch. The columns included a Phenogel 5-μm guard column (50 mm length, 7.8 mm i.d.) (Phenomenex, Torrance, CA), followed by a series of four Phenogel columns [5-μm particle size with pore sizes of 500, 100, 100 (500 and 8.0 mm i.d.), and 50 Å (300 and 7.8 mm i.d.), Phenomenex, Torrance, CA] that was connected to an evaporative light scattering detector (ELSD II A, Varex Corp., Burtonsville, MD). The detector was operated at the following conditions: adjusted temperature at 100 °C, heater temperature at 98.6 °C, exhaust temperature at 50.5 °C, ultra-high-purity nitrogen (99.999%) gas flow rate of 39 mL/min, a pressure of 7400 kg/m^2, range 20, and time constant of 0.5. The mobile phase, tetrahydrofuran (THF), was filtered with 0.45-μm HV disks (Millipore Corp., Bedford, MA) and degassed by sonication before use. The solvent was contained in an amber bottle under a nitrogen gas atmosphere and was delivered at a flow rate of 1.0 mL/min. Oil samples were accurately weighed (± 0.01 mg) diluted with THF to a concentration of approximately 1.5−2.0 mg of oil/mL in THF and filtered with 0.22-μm HV disks before injection. The HPSEC analyses were done in triplicate (Hansen and Artz, 1994; Hansen et al., 1994; Artz et al., 1997).

Polypropylene glycol (PPG) molecular weight standards of PPG triol (6000, 4100, 3000, and 1500), triolein (885.4), diolein (621.0), and monoolein (356.5) (Aldrich Chemical Co., Milwaukee, WI) were used to determine the relation between retention time and molecular weight (MW). The log of the MW of the PPG standards was plotted versus the retention time, T_r, and the response factors were estimated from the molecular weight:

$$\log (\mathrm{MW}) = 6.99 - (0.01)(T_r) \quad (1)$$

The molecular weights and percent components were estimated based on the method developed by Hansen and Artz (1994). The peak area analysis was performed on HPLC Method Manager Software (Rainin Instruments Co., Woburn, MA).

Supercritical Fluid Chromatographic Analysis. Each sample, including the internal standard, was accurately weighed (± 0.00001 g), dissolved, and brought to volume. The internal standard used was tricaprin (Sigma Chemical Co., St. Louis, MO), and the solvent was methylene chloride (Fisher Scientific, Fair Lawn, NJ) (Lu et al., 1993).

The supercritical fluid chromatograph used was a Lee Scientific model 501β (Lee Scientific, Inc., Division of Dionex, Salt Lake City, UT) with a Valco A90 injector (Houston, TX) with a 0.5-μL internal loop operated in a time-split mode. The capillary column used was a 20 m SB-methyl-100 (50 mm i.d., d_f = 0.25 mm). The stationary phase was 100% polymethyl siloxane. The mobile phase was SFE/SFC grade CO_2 (Air Products and Chemicals Inc., Allentown, PA). The separations were achieved using density programming (0.15 g/mL, asymptotic ramp to 0.64 g/mL, 1/2 rise time 15 min, end time 90 min.). The column temperature was 150 °C, and the FID 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 was collected and analyzed with a Hyundai 386SX PC and Baseline software (Waters Chromatography, Milford, MA) (Artz et al., 1997).

The chromatograms were integrated, and peak retention times and areas were obtained. Quantitative analysis of triacylglycerols was based on component concentrations that were calculated using the peak areas relative to the internal standard peak area. Component concentrations were calculated from the following equation: $C_c = C_i(A_c/A_i)$, where C_c is the concentration of the component, C_i is the concentration of the internal standard, A_c is the area of the component peak, and A_i is the area of the internal standard peak. The concentration of total triacylglycerols (TAGs) for each day was

Table 1. Chemical Analysis of Heated EPG-08 Soyate and EPG-00 Soyate[a]

sample	heating time (h)	temp (°C)	peroxide value (mequiv/kg)	free fatty acid value (% oleic)	acid value (mg of KOH/g of oil)	*p*-anisidine value (absorbance per g of oil)
EPG-08 soyate	unheated		2.36 ± 0.07	0.19 ± 0.00	0.26 ± 0.00	1.1 ± 0.4
EPG-08 soyate	12	195.7 ± 4.7	1.20 ± 0.03	0.38 ± 0.01	0.56 ± 0.04	144.5 ± 4.9
EPG-08 soyate	24	192.3 ± 5.0	0.65 ± 0.16	0.51 ± 0.01	0.98 ± 0.06	173.2 ± 3.1
EPG-08 soyate	36	189.3 ± 4.9	0.00 ± 0.00	0.71 ± 0.01	1.36 ± 0.08	191.7 ± 1.7
EPG-00 soyate	unheated		1.92 ± 0.08	0.19 ± 0.00	0.10 ± 0.01	1.6 ± 0.3
EPG-00 soyate	12	190.2 ± 6.5	1.65 ± 0.04	0.33 ± 0.01	0.36 ± 0.03	144.7 ± 4.1
EPG-00 soyate	24	196.6 ± 5.4	1.40 ± 0.03	0.51 ± 0.00	0.88 ± 0.05	188.1 ± 3.5
EPG-00 soyate	36	191.0 ± 5.9	1.14 ± 0.03	0.62 ± 0.02	1.17 ± 0.07	208.6 ± 2.6
EPG-00 soyate	48	192.8 ± 6.7	0.46 ± 0.05	0.79 ± 0.04	1.59 ± 0.03	195.4 ± 1.9

[a] Mean ± standard deviation.

Table 2. Physical Analysis of Heated EPG-08 Soyate and EPG-00 Soyate[a]

		color (1 in. height)			
sample	heating time (h)	yellow units	red units	dielectric constant (sensor reading)	absorbance at 234 nm (absorbance units)
EPG-08 soyate	unheated	2	0.0	0.00 ± 0.00	1678 ± 79
EPG-08 soyate	12	50	3.5	2.65 ± 0.02	2306 ± 409
EPG-08 soyate	24	70	7.0	4.73 ± 0.01	1868 ± 343
EPG-08 soyate	36	70	12.0	6.30 ± 0.02	2526 ± 915
EPG-00 soyate	unheated	2	0.6	0.00 ± 0.00	2185 ± 1
EPG-00 soyate	12	35	3.0	1.51 ± 0.01	2360 ± 2
EPG-00 soyate	24	50	5.0	2.89 ± 0.02	2715 ± 2
EPG-00 soyate	36	70	10.0	4.06 ± 0.04	3827 ± 5
EPG-00 soyate	48	70	16.0	5.14 ± 0.02	4053 ± 5

[a] Mean ± standard deviation.

an average of three replicates. The actual triacylglycerol concentrations were obtained by multiplying the SFC results by a response factor (R_F), which was equal to the concentration of the control (unheated) sample (as prepared) divided by the concentration as determined by SFC. For every heating period, the percentage of recovery of TAGs was calculated by dividing the actual concentration of these components in the sample by the actual concentration of TAGs in the control sample. Linear regression analysis was used to determine the slope from a plot of ln [TAG]/[TAG_o] versus time (h), where [TAG_o] is the initial TAG concentration, and [TAG] is the TAG concentration at any other time. From that analysis, the reaction rate constant and the standard deviation were determined (Artz et al., 1997).

RESULTS AND DISCUSSION

Each oil sample was heated in 12-h increments. Table 1 contains the changes in the peroxide value, the free fatty acid content, the acid value, and the *p*-anisidine value for both samples after heating for 12, 24, 36, and 48 h. Peroxides are not stable at frying temperatures, so a reduction in value upon heating was expected. Both samples showed similar physicochemical changes during heating. As expected, the FFA value and acid value increased as a result of hydrolysis and thermal oxidative degradation. Although the FFA, AV, and p-AV were comparable for both samples after 12 and 24 h of heating, the FFA, AV, and p-AV were greater for the EPG-08 soyate sample than for the EPG-00 soyate after 36 h of heating. Since the percent polymer content for the EPG-08 soyate sample exceeded the 20% polymer target end point after 36 h of heating, the heating was stopped for the EPG-08 soyate sample after 36 h. The heating was continued to 48 h for the EPG-00 soyate sample since the target end point of 20% polymer had not been reached. The FFA, AV, and p-AV values increased and were comparable, although slightly greater, to the EPG-00 soyate sample heated for 48 h but greater than for the EPG-08 soyate sample heated for 36 h. The FFA, AV, and p-AV data suggest that the EPG-00 soyate sample was slightly more stable than the EPG-08 soyate sample.

Table 2 contains the changes in the color, dielectric constant, and ultraviolet light absorbance for both samples after heating for 12, 24, 36, and 48 h. As expected, the color intensity, dielectric constant, and UV absorbance increased as a result of hydrolysis and thermal oxidative degradation. The color intensity and dielectric constant were greater for the EPG-08 sample than for the EPG-00 soyate after each heating period. The heating was continued to 48 h for the EPG-00 soyate sample, and the color intensity and the dielectric constant were comparable for the EPG-00 soyate sample heated for 48 h and for the EPG-00 soyate sample that was heated for only 36 h. The color and dielectric constant data suggest that the EPG-00 soyate sample was slightly more stable than the EPG-08 soyate sample. The UV absorption at a wavelength of 234 nm due to conjugated diene formation (Table 2) generally increased as frying time increased. The UV absorption was comparable for both oils after 12 h of heating, but it was greater for the EPG-00 soyate sample after 24 and 36 h of heating, which suggests that the EPG-00 soyate is slightly less stable than the EPG-08 soyate.

Summaries of the retention time, percent area, and average molecular weight of the monomeric and polymeric triacylglycerols, as determined by HPSEC, are presented in Tables3 and 4. Heated EPG-08 soyate reached a polymer content of >20% after 36 h of heating, while EPG-00 soyate reached a polymer content of >20% after 48 h of heating. The target end point of 20% for the polymer content was based on previous work that indicated that the concentration of polar compounds [27−28% polar material as determined by column chromatography (CC)] could be used to determine when an oil is considered excessively deteriorated and should be discarded (Billek et al., 1978; Paradis and Nawar,

Table 3. Size Exclusion Chromatography of Heated EPG-08 Soyate[a,b]

	heating time (h)			
	0 h (unheated)	12 h	24 h	36 h
oligomers, peak 1				
Tr (min)				33.00 ± 0.05
% component				6.41 ± 0.51
estimated MW				6450 ± 80
trimers, peak 2				
Tr (min)		35.48 ± 0.00	35.40 ± 0.03	35.38 ± 0.08
% component		1.58 ± 0.07	5.68 ± 0.51	9.17 ± 0.24
estimated MW		3740 ± 0	3800 ± 30	3810 ± 70
dimers, peak 3				
Tr (min)	36.74 ± 0.05	36.69 ± 0.05	36.63 ± 0.05	36.61 ± 0.03
% component	0.66 ± 0.01	5.57 ± 0.35	8.98 ± 0.36	12.17 ± 0.34
estimated MW	2830 ± 30	2860 ± 40	2900 ± 40	2910 ± 20
monomers, peak 4				
Tr (min)	39.53 ± 0.06	39.53 ± 0.06	39.45 ± 0.03	39.44 ± 0.06
% component	99.34 ± 0.01	92.85 ± 0.42	85.34 ± 0.87	72.25 ± 1.01
estimated MW	1530 ± 20	1530 ± 20	1550 ± 10	1560 ± 20

[a] Mean ± standard deviation. [b] The compounds eluted based on their size. The first group of compounds to elute (peak 1) was the triacylglycerol oligomers (comprised of four or more triacylglycerol monomers), followed by the triacylglycerol trimers, dimers, and monomers.

Table 4. Size Exclusion Chromatography of Heated EPG-00 Soyate[a]

	heating time (h)				
	0 h (unheated)	12 h	24 h	36 h	48 h
oligomers, peak 1					
Tr (min)		35.79 ± 0.03	35.70 ± 0.00	35.62 ± 0.03	35.68 ± 0.03
% component		0.57 ± 0.07	2.61 ± 0.02	5.69 ± 0.14	10.70 ± 0.21
estimated MW		2543 ± 19	2597 ± 0	2641 ± 19	2608 ± 19
trimers, peak 2					
Tr (min)	37.24 ± 0.00	37.14 ± 0.06	37.11 ± 0.03	37.05 ± 0.03	37.09 ± 0.03
% component	0.84 ± 0.09	3.07 ± 0.26	6.41 ± 0.21	8.42 ± 0.19	10.38 ± 0.21
estimated MW	1822 ± 0	1861 ± 27	1876 ± 14	1900 ± 14	1884 ± 14
dimers, peak 3					
Tr (min)	38.90 ± 0.06	38.89 ± 0.10	38.79 ± 0.03	38.78 ± 0.00	38.52 ± 0.23
% component	0.37 ± 0.05	0.48 ± 0.09	0.65 ± 0.10	0.72 ± 0.02	0.97 ± 0.09
estimated MW	1241 ± 18	1246 ± 28	1273 ± 9	1278 ± 0	1357 ± 71
monomers, peak 4					
Tr (min)	40.35 ± 0.03	40.28 ± 0.06	40.21 ± 0.00	40.15 ± 0.00	40.19 ± 0.03
% component	98.79 ± 0.14	92.41 ± 0.56	86.70 ± 0.65	81.33 ± 0.56	73.81 ± 0.22
estimated MW	889 ± 7	904 ± 13	919 ± 0	931 ± 0	923 ± 7

[a] Mean ± standard deviation.

1981a,b). Investigators have correlated the percentage of polar compounds in the heated oil to the concentration of polymeric material (Hussain et al., 1991; Billek et al., 1978), and ~27−28% polar material corresponds to 20% polymer. With the advancement in column performance (reduction in particle size and increased rigidity) for HPSEC, HPSEC is considered a much more efficient (much faster and more reproducible) method of analysis for heated oils than the percent polar compounds analysis using CC (Hussain et al., 1991; White and Wang, 1986), so 20% polymer was selected as the target end point.

Figure 1 is an overlay chromatogram of the SFC separations of EPG-08 for the unheated and 36-h samples, while Figure 2 is an overlay chromatogram of the SFC separations for EPG-00 for the unheated and 48-h samples. The chromatograms of the unheated and heated samples at the end of each heating period for each sample (36 h for EPG-08 soyate and 48 h for EPG-00 soyate) were used for the comparison. On the basis of the percent polymer determination, the EPG-08 soyate sample heated for 36 h and the EPG-00 soyate sample heated for 48 h have undergone comparable amounts of degradation. That is, both samples had 20% or more TAG polymer at the end of their respective heating period (36 h for EPG-08 soyate and 48 h for EPG-00 soyate) and were roughly equivalent in terms of the amount of thermal oxidative degradation that had occurred. The group of peaks in Figure 1 (retention time = ~50−73 min) represent triacylglycerols (TAGs) with different levels of propoxylation. For example, the component eluting at approximately 55 min contains one more molecule of propylene oxide than the oligomer or component eluting at ~52 min. The SFC analysis indicated that the concentration of unoxidized TAGs decreased with heating time for both oil samples. The sharp peak in Figure 2 (eluting at ~27 min) is the internal standard tricaprin. The component eluting at ~51 min represents TAGs with only C18 fatty acids, while the component eluting at ~48 min represents TAGs with two C18 fatty acids and a single C16 fatty acid. Since the stationary phase used was 100% polymethylsiloxane, separation occurred only the basis of number of carbon atoms and not the number of double bonds.

Supercritical fluid chromatography (SFC) provides much greater specificity than HPSEC for the direct determination of triacylglycerol (TAG) concentration. In

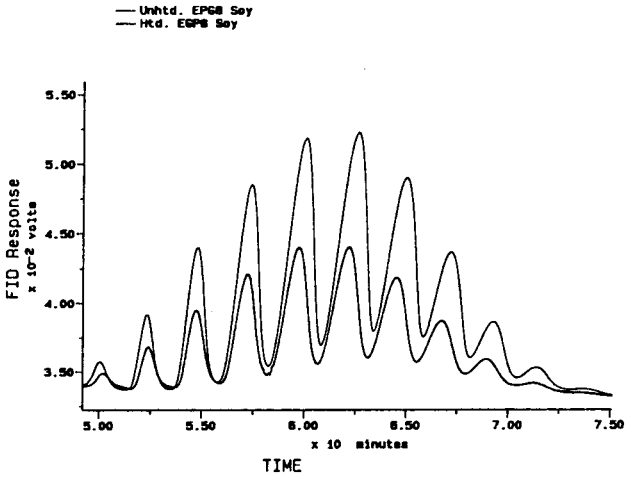

Figure 1. Overlay capillary supercritical fluid chromatogram of two samples, day 0 (unheated) and day 3 (36 h of heating) of EPG-08 soyate (soy oil-based potential fat substitute). The upper chromatogram is the unheated sample (thin line, unheated EPG8 soy), while the lower chromatogram (thick line, heated EGP8 soy) is the sample heated for 36 h. The 11 components eluting from ~50 to 74 min are propoxylated triacylglycerols with soybean oil fatty acids that differ by one propylene oxide group, e.g., the component eluting at ~52 min has one less propylene oxide group per molecule than the component eluting at ~55 min.

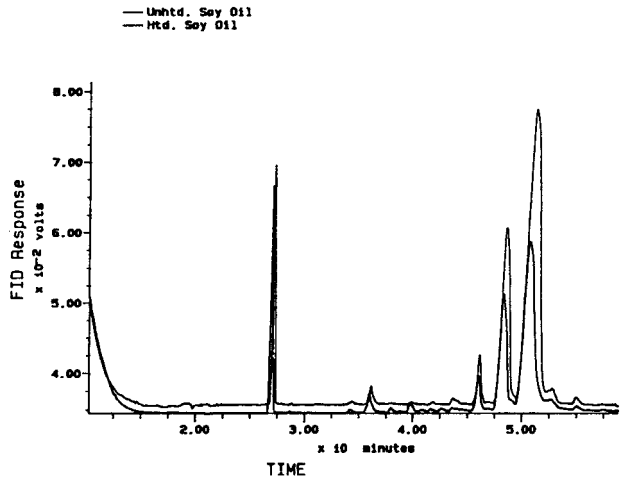

Figure 2. Overlay capillary supercritical fluid chromatogram of an unheated sample and a heated sample (4 day or 48 h of heating) of EPG-00 soyate (transesterified soybean oil). The upper chromatogram is day 0 or an unheated sample (thin line, unheated soy oil), while the lower is day 4 or 48 h (thick line, heated soy oil). The internal standard (tridecanoin) eluted at approximately 27 min. The components representing triacylglycerols (TAGs) containing two C16 and one C18 fatty acids eluted at ~46 min, while the components representing triacylglycerols (TAGs) containing one C16 and two C18 fatty acids eluted at ~48 min, and the components representing triacylglycerols (TAGs) containing three C18 fatty acids eluted at ~51 min.

contrast to HPSEC, triacylglycerols differing by a single double bond (e.g., OLL and LLL) can be separated with capillary SFC (Artz, 1993; Lee and Markides, 1990) given the appropriate stationary phase. Therefore, any change in the TAG structure induced as a result of oxidation (cleavage of a 3−12 carbon fraction, formation of a hydroxy fatty acid from the addition of a hydroxy radical, or even cross-linking via a carbon−carbon bond of two adjacent fatty acids on the same triacylglycerols) would result in a substantial change in the retention time with capillary SFC, unlike HPSEC where all of the altered triacylglycerols were coeluted in the monomer peak. Therefore, unlike the HPSEC monomer fraction, the compounds eluting in the SFC chromatograms from 49 to 75 min (Figure 1) and from 45 to 56 min (Figure 2) represent completely unaltered triacylglycerols.Summaries of the concentrations of both samples including recoveries of the TAGs are presented in Table 5. After heating, the percentage of unoxidized or unaltered TAGs contained in the EPG-08 soyate sample was reduced to 53.6% after 3 days of heating, while the percentage of unaltered TAG in the EPG-00 sample was reduced to 56.1% after 4 days of heating. In comparison, after 36 h of heating, the monomeric TAG peak as determined by HPSEC was 72.25%, while the monomeric TAG content as determined by SFC was only 53.6%. The greater HPSEC value reflects the inclusion of altered TAGs in the monomeric TAG peak, unlike the SFC analysis.

Table 5. SFC Analysis of EPG-08 Soyate and EPG-00 Soyate after Heating[a]

sample	heating time (h)	TAG concn[b] (mg/mL)	% TAG in sample	% recovery
EPG-08 soyate	unheated	22.50 ± 0.59[c]	90.1	100.0
EPG-08 soyate	12	18.51 ± 1.79	74.0	82.2
EPG-08 soyate	24	16.50 ± 1.61	65.9	73.3
EPG-08 soyate	36	12.06 ± 0.65	48.2	53.6
EPG-00 soyate	unheated	9.88 ± 0.07[c]	98.6	100.0
EPG-00 soyate	12	8.53 ± 0.42	85.1	86.3
EPG-00 soyate	24	7.61 ± 0.13	76.0	77.1
EPG-00 soyate	36	6.46 ± 0.10	64.4	65.4
EPG-00 soyate	48	5.54 ± 0.01	55.4	56.1

[a] The initial concentration (mg/mL) for EPG-08 soyate was 25.01 ± 0.03, while the initial concentration for EPG-00 soyate was 10.01 ± 0.02. [b] The concentration of the triacylglycerol (TAG) components were calculated from the SFC data and corrected by the response factor (RF). [c] Mean ± standard deviation.

The reaction rate constant (apparent first-order) for loss of EPG-00 soyate during heating was 0.0126 ± 0.0003 h^{-1} (correlation coefficient = 0.99), while the reaction rate constant for the loss of EPG-08 soyate during heating was 0.0166 ± 0.0017 h^{-1} (correlation coefficient = 0.90). The results from the majority of the analyses, including the chromatographic analyses, suggest that the EPG-00 soyate (or transesterified soybean oil) is slightly more stable than EPG-08 soyate.

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