Nuclear Magnetic Resonance and Gas Chromatography/Mass Spectroscopy Analysis of the Nonvolatile Components Produced during Heating of Oleic Acid Esterified Propoxylated Glycerol, a Fat Substitute Model Compound, and Trioleylglycerol

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Oleic acid esterified propoxylated glycerol (EPG-08 oleate) and trioleylglycerol were heated separately (192 ± 8 °C for 12 h/day) until the polymer content was ≥20% and examined. Supercritical fluid fractionation (SFF) produced a monomer fraction of 98.3% purity and a dimer fraction of 90.8% purity for heated EPG-08 oleate and comparable fractions for triolein. Carbon-13 NMR analysis of the fractionated dimer (SFF-D) oil samples indicated peaks at approximately 107−108, 67−68, and 23.8 ppm that were present in only the SFF-D samples. The presence of these peaks in both samples indicated that the presence of the oxypropylene backbone was not necessary for the formation of the bonds corresponding to these peaks. The oxypropylene backbone profile was the same for the day 0 and the SFF-D sample. Gas chromatography/mass spectrometry of dimeric fatty acid methyl esters indicated that the fatty acid portion of the molecule was involved in dimer formation for both oil samples.

Keywords: *Oleic acid esterified propoxylated glycerol; triolein; trioleylglycerol; fat substitutes*

INTRODUCTION

Oxidation and heating studies have been conducted on methyl oleate and linoleate (Christopoulou and Perkins, 1989a), trilinolein, triolein, and tristearin (Chang et al., 1978; Paulose and Chang, 1978; Neff et al., 1990), and mixtures of unsaturated and saturated triacylglycerols (TAGs) (Husain et al., 1991), as well as the fat-based fat substitute Olestra (Gardner and Sanders, 1990; Sanders et al., 1990). In addition, heating studies simulating deep fat frying have been conducted on pure triolein (Chang et al., 1978). The iodine value for triolein decreased, indicating loss of double bonds as a result of oxidation and polymerization. The heated triolein contained 3.4% noncyclic dimers joined by C−C bonds, 0.3% trimers joined by C−C bonds, and 6.2% dimers and trimers partially or wholly joined by C−C and C−O bonds.

Oils used in deep fat frying typically contain >96% TAG (monomer), <4% polar component, 0.5% polymeric material, 0.02% free fatty acid (FFA), 0.01% oxidized FFA, and 0−7 ppm soaps prior to heating (Blumethal and Stier, 1991). As the oil is heated, the TAG concentration decreases and the concentrations of polar compounds, polymeric material, FFA, oxidized FFA, and soaps increase.

The primary objective of this research was to characterize the chemical changes that occurred in the fat substitute model compound, oleic acid esterified propoxylated glycerol, upon heating at deep fat frying temperatures. This included identification of the location and type(s) of chemical bonds that were formed in one of the major nonvolatile decomposition products, dimeric TAGs, which were formed from both oleic acid esterified propoxylated glycerol and another closely related model compound, trioleylglycerol, upon heating.

MATERIALS AND METHODS

Oil Sample Preparation. Model compounds of approximately 4 L each of oleic acid esterified propoxylated glycerol (EPG-08 oleate) and triolein were prepared by ARCO Chemical Co. (Newtown Square, PA). No antioxidants were added to either model compound. Capillary gas chromatographic analysis indicated the percentage of oleic acid (as a percentage of the total fatty acids) in the triolein sample was 99.4 ± 0.1%, while the percentage of oleic acid in the EPG-oleate sample was 97.8 ± 0.3%. The designation "08" in EPG-08 oleate indicates that the initial mole ratio was 8 mol of propylene oxide/mol of glycerol. Each sample was heated in a deep fat fryer (Model F175A, Intedge Industries, Inc., Whippany, NJ) at 190 °C for 12 h per day, until the polymer concentration was ≥20%. After heating, samples were collected and then fractionated with an ISCO supercritical fluid extraction (SFE) System 2100 (Isco Inc., Lincoln, NE) (Hansen and Artz, 1994).

Supercritical Fluid Fractionation (SFF)/High-Performance Size Exclusion Chromatography (HPSEC). To purify the dimeric fraction of the heated oil samples for subsequent analysis by gas chromatography/mass spectrometry (GC/MS) and nuclear magnetic resonance (NMR), samples of day 2 (24 h) EPG-08 oleate and day 4 (48 h) triolein were dynamically and sequentially fractionated with the ISCO SFE system (Hansen and Artz, 1994). Fractionation times were monitored and the amount extracted was determined by direct gravimetric analysis after each extraction. The fractionation conditions for each oil sample were as follows. For the EPG-08 oleate sample, the first extraction to remove the monomer fraction was at 306 atm for 120 min, while the second extraction to isolate the dimer TAG fraction was as 425 atm

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S0021-8561(97)00614-6 CCC: $14.00

for 90 min. For the triolein sample, the first extraction was at 272 atm for 120 min, while the second extraction to isolate the dimer TAG fraction was at 306 atm for 120 min.

For the HPSEC analysis, an autosampler (Model 728, Alcott Chromatography, Norcross, GA) and four Phenogel columns [particle size of 5 μm, pore sizes of 500 Å (500 mm × 8 mm i.d.), 100 Å (2 × 500 mm × 8 mm i.d.), and 50 Å columns (300 mm × 7.8 mm i.d.)] were used in series. The conditions for the Varex IIA evaporative light scattering detector are reported elsewhere (Hansen and Artz, 1994).

A formula was determined for estimating the molecular weight (MW) based on the retention volume (V_r). Polypropylene glycol triol (PPGT) standards (Aldrich Chemical Co., Milwaukee, WI) with MWs of 6000, 4100, and 3000, in addition to triolein, diolein, and monoolein, were used as MW standards. The log of the MW of the standards was plotted vs the V_r, and eq 1 was derived from the plot. The MWs of the HPSEC resolved components of the heated EPG-08 oleate and triolein samples were estimated from the V_r with

$$\log \mathrm{MW} = 6.99 - 0.0991 V_r \quad (1)$$

The response of the Varex IIA detector was not linear with respect to the MW. The response factor (R_f) was determined by plotting the area/concentration ratio vs the MW of the standards:

$$R_f = (1.23 \times 10^6) + 30.2(\mathrm{MW}) - 0.0220(\mathrm{MW})^2 \quad (2)$$

The percent purity was determined from the HPSEC analysis (Hansen and Artz, 1994). All samples were done in triplicate.

Nuclear Magnetic Resonance Spectrometry (NMR). ^{13}C NMR, ^{1}H NMR, distortionless enhancement via polarization transfer (DEPT), and ^{1}H−^{13}C heteronuclear correlation spectroscopy (HETCOR) spectra were recorded on a Varian Unity NMR spectrometer operating at 399.952 MHz for proton and 100.577 MHz for carbon. Approximately 50−100 mg of the monomer and dimer fractions from the day 2 (24 h) EPG-08 oleate sample, the monomer and dimer fractions from the day 4 (48 h) triolein sample, and the day 0 (unheated, unfractionated) samples for the triolein and the EPG-08 oleate were dissolved in 0.6 mL of $CDCl_3$ with 1% v/v tetramethylsilane (TMS) as the internal standard (Aldrich Chemical Co., Milwaukee, WI) in a 5 mm Wilmad 528-PP NMR tube. Samples of Polyol 550, the product formed by the propoxylation of glycerol, but prior to the esterification of fatty acids, were similarly prepared for analysis. Spectra were collected at 20 °C. Longitudinal relaxation times (T_1) were not determined. ^{13}C spectra were collected with 512 transients, DEPT with 128 transients, and proton spectra with 32 transients.

Gas Chromatography/Mass Spectrometry (GC/MS). Dimer and monomeric TAG fractions of the day 2 (24 h) EPG-08 oleate sample and the day 4 (48 h) triolein sample were transferred from the NMR tubes with Pasteur pipets into a 25 mL test tube, and the solvent was evaporated with nitrogen. Each oil sample (50−100 mg) was transesterified according to the procedures of Metcalfe et al. (1966), with the following changes: a 25 mL test tube was used instead of a 50 mL volumetric flask, BF_3−methanol (MeOH) (12%, Aldrich Chemical Co., Milwaukee, WI) was purchased instead of prepared on site, a boiling water bath was used for heating, petroleum ether was removed with a Pasteur pipet and evaporated with nitrogen to dryness, and the sample was resuspended in 20 μL of petroleum ether. A number of transesterification procedures were evaluated. The first procedure used BF_3 (12%) in methanol (Metcalf et al., 1966); the second used trimethylsulfonium hydroxide in methanol (Butte, 1983); the third used sodium methoxide in methanol (Christie, 1989); and the fourth method examined required BF_3 (6%) in methanol (O'Keefe et al., 1993). The BF_3−methanol method of Metcalfe et al. (1966) appeared to be the best among those examined for the transesterification of all of the dimeric TAG samples, including the EPGs.

A 5890 Series II capillary GC (Hewlett-Packard, Naperville, IL) with a DB-5ht column (30 m × 0.25 mm i.d. × 0.1 μm film thickness (d_f), J&W Scientific, Folsom, CA) connected to a 5970 mass selective detector (MSD) (Hewlett-Packard) was used for the separation, quantitation, and identification of the fatty acid methyl esters (FAMEs). Direct injection through a split/splitless liner (Part 19251-60540, Hewlett-Packard) was used. The injection temperature was 310 °C with a temperature program of 250 °C (2 min) to 300 °C at 10 °C/min. The column pressure was 0.442 atm with helium as the carrier gas. A split ratio of 1:5 was used. Injection volume was 0.1 μL. A commercial sample of dimer fatty acids (Empol 1008, Henkel Corp., Emery Group, Cincinnati, OH) was methylated and used as a FAME standard to determine the retention times for the dimeric FAMEs.

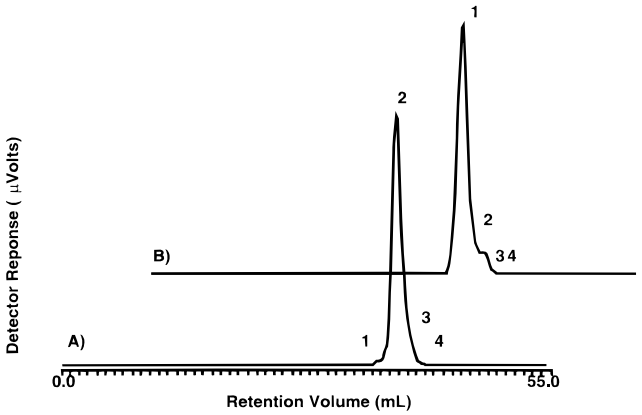

Figure 1. HPSEC of fractionated EPG-08 oleate (day 2, 24 h): (A) monomer fraction collected at 4500 psi (306 atm) [peak 1 = dimeric TAG, peak 2 = monomeric TAG, and peaks 3 and 4 = low molecular weight (LMW) products]; (B) dimer fraction collected at 6250 psi (425 atm) after 4500 psi (306 atm) [peak 1 = dimeric TAG, peak 2 = monomeric TAG, and peaks 3 and 4 = LMW products]. The monomer fraction purity was 98.3%, and the dimer fraction purity was 90.8%.

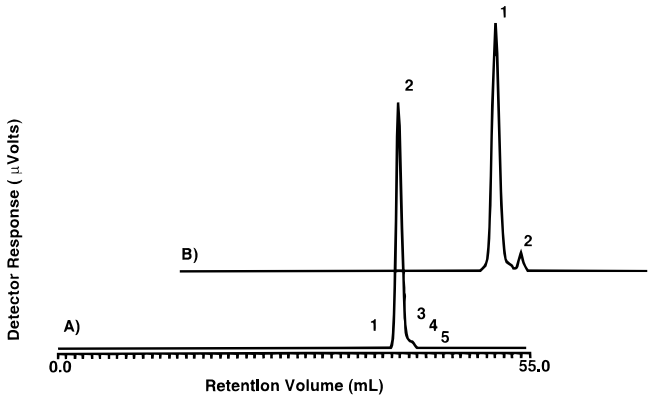

Figure 2. HPSEC of fractionated triolein (day 4, 48 h): (A) monomer fraction collected at 4000 psi (272 atm) [peak 1 = dimeric TAG, peak 2 = monomeric TAG, peak 3 = diacylglycerol (DAG), and peaks 4 and 5 = low molecular weight (LMW) products]; (B) dimer fraction collected at 4500 psi (306 atm) after 4000 psi (272 atm) [peak 1 = dimeric TAG and peak 2 = monomeric TAG]. The monomer fraction purity was 98.8%, and the dimer fraction purity was 93.4%.

RESULTS AND DISCUSSION

Supercritical Fluid Extraction (SFE) Fractionation. Monomer and dimer TAG fractions of heated samples of triolein and EPG-08 oleate were isolated with a SFE system, and the purity of the isolated fractions was determined with HPSEC (Figures 1 and 2). High percentage recoveries for triolein were more difficult to obtain than with the EPG sample, due to the smaller MW difference between the monomer and dimers for the triolein, as compared to the EPG sample. The collected

Table 1. Spectral Data for the ^{13}C Spectrum of Polyol 550 (Propoxylated Glycerol)

peak no.	ppm[a]	intensity[b]	assignment[c]
80	18.665	9.916	PO_3
81	18.634	11.249	PO_3
82	18.619	11.663	PO_3
83	18.574	12.991	PO_3
84	18.513	17.142	PO_3
85	18.430	34.482	PO_3
86	18.384	61.711	PO_3
87	18.285	19.700	PO_3
88	18.240	14.655	PO_3
89	18.217	13.384	PO_3
90	18.179	21.161	PO_3
91	18.111	26.658	PO_3
92	17.231	8.008	PO_1
93	17.216	10.023	PO_1
94	17.201	10.472	PO_1
95	17.163	12.028	PO_1
96	17.011	50.263	PO_1
97	16.829	24.161	PO_1
98	16.799	5.318/21.100	PO_2
99	16.746	29.532	PO_2
100	16.662	7.128/11.598	PO_2
101	16.624	7.625/3.641	PO_2
102	16.571	6.312	PO_2
103	16.488	5.254	PO_2

[a] Relative to the internal standard TMS. [b] Intensity is determined during DEPT experiment. [c] Peaks that could not be assigned were not included. [d] PO refers to the propylene oxide group and the subscript refers to the location of the PO group relative to its glycerol attachment; i.e., PO_1 is the propylene oxide group attached directly to glycerol.

monomer and dimer fractions (comingled after numerous extractions) of EPG-08 oleate were 98.3 and 90.8% pure, respectively. The collected monomer and dimer fractions (comingled after numerous extractions) of triolein were 98.8 and 93.4% pure, respectively.

NMR Analysis. The ^{13}C NMR spectrum for Polyol 550 (propoxylated glycerol with an initial reaction mixture mole ratio of 8 mol of propylene oxide/mol of glycerol) is summarized in Table 1. The DEPT ^{13}C NMR spectrum for Polyol 550 was also collected and analyzed (figure not shown). DEPT experiments are particularly useful for peak assignments since one can determine multiplicities (CH, CH_2, and CH_3), and DEPT improves sensitivity by decreasing relaxation delay times and enhancing signal intensity (Wollenburg, 1991). Methine resonances are present at 78.3−78.0, 76.5−76.3, 75.7−75.2, 74.9−74.5, and 67.0−65.4 ppm. Methylene resonances appear at 77.2−77.0, 75.9−75.2, and 74.3−70.9 ppm. Peak assignments for this complex region have been previously reported (Schilling and Tonelli, 1986). It may not be necessary to determine all of the individual peak assignments for the oxypropylene backbone, if the type and number of bonds or absorbances corresponding to the oxypropylene backbone do not change between the unheated sample of fatty acid esterified propoxylated glycerols (EPGs) and the fractionated EPG dimer (SFF D) sample. Two groups of methyl carbons appeared in the Polyol 550 spectrum at 18.7−18.1 and 17.2−16.5 ppm (Table 1). The ratio of the methyl groups at ≈18−17 ppm was 1:1.4 or ≈2:3. The importance of this ratio will be discussed later.

The ^1H spectrum of Polyol 550 was recorded (figure not shown). There are peaks at 3−4 ppm representing a possible methylene α to a hydroxyl or an ether, a methine α to a hydroxyl or ether, or a methyl α to an ether or a hydroxyl. The resonances at 1.2−1.0 ppm

Table 2. Spectral Data for the ^{13}C Spectrum of EPG-08 Oleate (Unheated, Day 0)

peak no.	ppm[a]	intensity[b]	assignment[c]
1	173.181	*d*	C_1
2	173.120	*d*	C_1
3	129.820	39.895	C_{10}
4	129.585	36.318	C_9
43	34.421	27.341	C_2 (β)
44	34.330	0.428/1.458	C_2 (α)
45	31.781	34.458	$\omega 3$ (C_{16})
46	29.634	41.922	MeE[e]
47	29.573	36.340	MeE
48	29.399	37.691	MeE
49	29.202	55.629	MeE
50	29.080	33.255	MeE
51	28.989	41.997	MeE
52	28.974	42.018	MeE
53	27.077	47.381	*cis* C_{11}
54	27.032	38.279	*cis* C_8
55	24.870	34.411	C_3
56	22.564	31.317	$\omega 2$ (C_{17})
57	17.208	13.925	PO_1
58	17.110	9.452	PO_2
59	16.655	22.943	PO_3
60	14.007	29.285	$\omega 1$ (C_{18})

[a] Relative to internal standard TMS. [b] Intensity determined during DEPT experiment. [c] Peaks that could not be assigned were not included. [d] C_1 is a quaternary carbon and does not show up during a DEPT experiment. [e] MeE = methylene envelope (C_{4-7} and C_{12-15}) for oleic acid TAG.

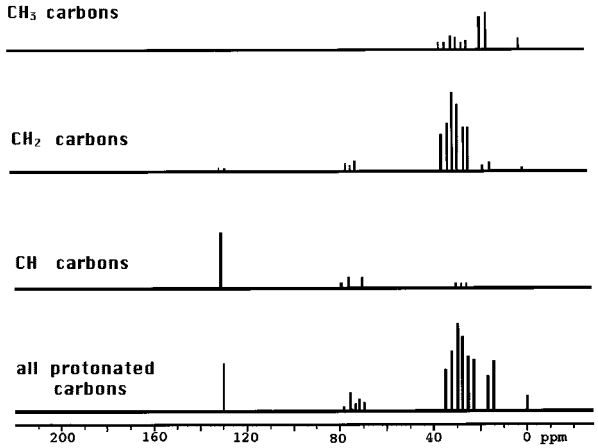

Figure 3. DEPT (distortionless enhancement by polarization technique) ^{13}C NMR spectrum of an unheated sample of oleic acid esterified propoxylated glycerol (EPG-08 oleate).

are indicative of CH_3 groups (Henderson et al., 1994). Integration of the areas indicates an area ratio of the peaks at 3.93 to 3.74−3.18 to 1.13 ppm was 1.0:10.1:7.9.

Table 2 contains a summary of the ^{13}C spectrum of the unheated or day 0 EPG-08 oleate sample. The DEPT spectrum for EPG-08 oleate is shown in Figure 3. There is a single carbonyl resonance at 173.2 with a shoulder at 173.1 ppm. The peak at 173.2 is indicative of the C_1 (1-,3-) (Ng, 1985; Wollenburg, 1990; 1991; Gunstone, 1991a,b, 1993a; Medina et al., 1994). However, there is only one major carbonyl peak, which suggests similar environments for each ester bond (C_1) in the EPG-08. This may have been due to an averaging effect among the many structures of widely varying propylene oxide chain lengths which would blur any distinctions.

Diacylglycerols (DAGs) contain resonances at 71−61 ppm (Sacchi et al., 1993). However, there were no resonances downfield from the TAG carbonyl as occurs with DAGs (Medina et al., 1994).

The olefinic peaks C_{10} and C_9 are at 129.8 and 129.6 ppm, respectively. Again, there are not two resonances (1,3- and 2-) at either olefinic carbon which would be expected of regular TAGs (Ng, 1984; Gunstone, 1990a,b, 1991a,b; Wollenburg, 1990, 1991; Husain et al., 1993). The complex mixture of peaks in the 79−69 ppm range for Polyol 550 and day 0 EPG-08 oleate indicate similar methine peak(s) at 78.4 ppm. There were two methine peaks at 76.5−76.3 ppm in the Polyol 550 that were not present in the day 0 EPG-08 oleate. The profile of the large number of methine and methylene peaks from 75.8 to 74.8 ppm in the Polyol 550 was similar to the profile of the day 0 EPG-08 oleate peaks in the range from 75.5 to 74.8 ppm (after esterification). Although there may have been a small change in the structure of the Polyol 550 component of EPG-08 upon esterification, one should not infer anything about EPG-08 stability from the Polyol 550 structure.

The methylene peaks in the Polyol 550 ranged from 74.3 to 70.9 ppm and from 74.3 to 71.2 ppm in day 0 EPG-08 oleate (data not shown). The methine peaks at 67−65 ppm in the Polyol 550 are not present in the day 0 EPG-08 oleate. However, the peaks from 69.5 to 68.9 ppm in day 0 EPG-08 oleate were methines (data not shown). For the day 0 EPG-08 oleate sample, the methylene peak at 34.4 ppm was indicative of a C_2 (2-), and the small methylene peak at 34.3 ppm may have been for C_2 (1,3-). The methylene peak at 31.8 ppm was for the ω3 carbon. The group of peaks from 29.6 to 29.0 ppm was indicative of the methylene envelope (MeE) (C_{4-7} and C_{12-15}) and is difficult to assign. There were seven major peaks in this region. The two methylene peaks at 27.1 and 27.0 ppm were the allylic carbons cis C_{11} and C_8, respectively. The C_3 methylene peak is at 24.9 ppm, and the ω2 methylene peak is at 22.6 ppm (Gunstone, 1990a,b, 1991b, 1993a,b; Wollenburg, 1991; Husain et al., 1993).

For the day 0 EPG-08 oleate sample, methyl peaks were observed at 17.2−17.1, 16.7, and 14.0 ppm. The methyl peak at 14.0 ppm is the ω1 of the fatty acid chain (Gunstone, 1990a,b, 1991b, 1993a,b; Wollenburg, 1991; Husain et al., 1993). The shapes of the methyl peak(s) at 17 ppm for the unheated or day 0 EPG-08 oleate sample were similar to the methyl peak(s) at 17 ppm in the Polyol 550, suggesting only a slight downfield shift upon esterification (Table 1). The peak(s) at 16.6 ppm in day 0 EPG-08 oleate shifted upfield during esterification as compared to their corresponding peak(s) 18.5−18 ppm in the Polyol 550, indicating the peak at 16.6 ppm in the day 0 EPG-08 oleate was the oxypropylene group closest to the fatty acid chain (PO_3), while the two peaks at ∼17 ppm were the oxypropylene groups closest to the glycerol carbons ($PO_{1,2}$).

The peak area ratio for day 0 EPG-08 oleate at 17−16 ppm (oxypropylene backbone) to 14 ppm (fatty acid) was about 3.8:1 (or 11.4:3). One would expect an oxypropylene backbone to fatty acid methyl carbon ratio of 8:3 for the EPGs, since the mole ratio of the oxypropylene to FA was 8:3. Thus, complete esterification of the propoxylated glycerol with oleic acid was not obtained. This observation corresponds with the IR data, which suggest the oil did contain free OH groups, and the SFC analysis, which indicated the oil contained ≈10% DAG or diester components (Artz et al., 1997).

The peak area ratio of the oxypropylene backbone methyls for day 0 EPG-08 oleate at ∼17.2−16.6 ppm was approximately 1.9:1. It is most likely that the ratio of 17.2/16.6 ppm methyl peaks reflects the ratio of five diether oxypropylene units to three monoesterified oxypropylene units found in EPG-08 oleate. Mass spectral analysis of the relative rates at which propylene oxide adds to glycerin indicates a ratio of 3:2:3 (Cooper and Polley, 1995) (for carbons 1−3 of the glycerol), but no confirming (or contradictory) evidence could be extracted from the NMR data.

For the ^{1}H spectrum of unheated day 0 EPG-08 oleate, the olefinic and hydroxyl protons were observed in the region of 5.6−5.0 ppm (figure not shown). The larger peak at 5.35 ppm is expected to be the olefinic peak, while the smaller peak at 5.0 ppm may be a hydroxyl proton. Theoretically, there should be six olefinic hydrogens per TAG, and the free OH group precentage should be <10% of the TAG concentration (as determined by IR and SFC). In a regular long-chain fatty acid TAG, the 2-glycerol proton should be at 5.25 ppm and the 1,3-glycerol protons are at 4.3 and 4.1 ppm (Wollenburg, 1991; Aursand et al., 1993; Henderson et al., 1994). These peaks are absent in the day 0 EPG-08 oleate.

The peak in the proton NMR spectra of the unheated EPG-08 oleate sample (day 0) (figure not shown) at 3.53 is both methine and methylene, while the peak at 3.42 ppm is solely methylene (Bruch et al., 1985). The peak at 2.29 ppm is a methylene α to a carbonyl (−CO−C**H**$_2$−) (Wollenburg, 1991; Aursand et al., 1993; Henderson et al., 1994). The peak at 2.02 ppm is for the allylic protons (−HC═CH−C**H**$_2$−). The peak at 1.61 ppm is indicative of a methylene β to a carbonyl (−COCH$_2$C**H**$_2$−) (Ng and Koh, 1988; Gunstone, 1993b). The group of peaks from 1.30 to 1.13 ppm is the methylenes in the oxypropylene and fatty acid chain (−C**H**$_2$−). The methyl peak is at 0.88 ppm (C**H**$_3$−).

Further discussion will focus on spectral differences or peaks that were not previously identified in either the Polyol 550 (Table 1) or the unheated day 0 EPG-08 oleate sample (Table 2).

The ^{13}C spectrum for the fractionated monomer (SFF M) day 2 (24 h) EPG-08 oleate sample has a carbonyl peak at 173.2 ppm, similar to the peak in the day 0 sample (figure not shown). The olefinic peaks at 129.8 (C_{10}) and 129.6 ppm (C_9) are also similar to the peaks for the day 0 or unheated EPG-08 oleate sample. There is a methine peak at 107.7 ppm that was not present in the day 0 sample. This peak may be due to the small amount of dimeric EPG-08 oleate remaining in the sample.

The supercritical fluid fractionated monomer (SFF M) EPG-08 oleate isolate sample purity (as determined by HPSEC) was 98.3%. The peak shapes and C−H assignments through the use of DEPT are similar for day 0 EPG-08 oleate and SFF M EPG-08 oleate samples (figure not shown). However, there are two methylene peaks at 67.8 and 67.4 ppm that were not present in the day 0 sample. These peaks may have also been due to the presence of a small amount of dimer in the SFF M sample.

Most of the peaks in the 35−0 ppm region are the same for day 0 and SFF M, except for a peak at 23.8 ppm (figure not shown). This methylene peak is between the C_3 and ω2 (C_{17}) of the FA chain. This peak was not present in the day 0 sample. There is also only one peak, and not two, at 34.4 ppm. There is a larger concentration of trans isomer in the SFF M than in the day 0 sample, as expected. A methylene peak at 32.4 ppm is indicative of trans C_8, C_{11} (Gunstone, 1993a; Aursand et al., 1993), although present in small amounts.

Table 3. Spectral Data for the ^{13}C NMR Spectrum of the EPG-08 Oleate Dimer Fraction (SFF D, Day 2, 24 h)

peak no.	ppm[a]	intensity[b]	assignment[c]
1	173.302	*d*	C_1
2	173.257	*d*	C_1
3	129.866	33.704	C_{10}
4	129.623	33.035	C_9
59	34.450	25.873	C_2 (β)
60	32.008	3.326	C_2 (α)
61	31.803	30.454	$\omega 3$ (C_{16})
63	29.657	38.618	MeE[e]
64	29.604	33.582	MeE
65	29.422	35.366	MeE
66	29.217	53.041	MeE
67	29.103	32.868	MeE
68	29.073	38.387	MeE
69	29.019	36.651	MeE
70	28.997	3.692	MeE
71	27.100	43.789	*cis* C_{11}
72	27.062	34.761	*cis* C_8
74	24.900	29.860	C_3
76	22.587	28.718	$\omega 2$ (C_{17})
77	17.223	15.195	PO_1
78	17.155	10.581	PO_1
79	17.132	10.537	PO_2
80	16.677	24.152	PO_3
81	14.037	29.255	$\omega 1$ (C_{18})

[a] Relative to internal standard TMS. [b] Intensity was determined during DEPT experiment. [c] Peaks that could not be assigned were not included. [d] C_1 is a quaternary carbon and does not show up during a DEPT experiment. [e] MeE = methylene envelope (C_{4-7} and C_{12-15}) for oleic acid TAG.

Table 4. Spectral Data for ^{13}C and DEPT NMR Spectrum of Triolein (Day 0)

peak no.	ppm[a]	intensity[b]	assignment
1	173.143	*c*	C_1 (α)
2	172.734	*c*	C_1 (β)
3	129.904	27.803	C_{10}
4	129.608	27.317	C_9 (α)
5	129.585	16.359	C_9 (β)
6	68.792	12.873	G_2 (β)
7	62.010	24.270	$G_{1,3}$ (α)
8	34.110	13.389	C_2 (β)
9	33.943	24.879	C_2 (α)
10	31.857	29.427	$\omega 3$
11	29.710	40.180	MeE[d]
12	29.649	29.007	MeE
13	29.482	33.902	MeE
14	29.277	56.355	MeE
15	29.148	20.775	MeE
16	29.126	26.833	MeE
17	29.057	30.706	MeE
18	29.027	24.836	MeE
19	28.989	10.855/4.304	MeE
20	28.890	2.363	MeE
21	27.153	42.602	*cis* C_{11}
22	27.108	38.118	*cis* C_8
23	24.817	17.591	C_3 (β)
24	24.779	25.456	C_3 (α)
25	22.640	28.616	$\omega 2$
26	14.060	27.219	$\omega 1$

[a] Relative to internal standard TMS. [b] Intensity was determined during DEPT experiment. [c] C_1 is a quaternary carbon and does not show up during a DEPT experiment. [d] MeE = methylene envelope (C_{4-7} and C_{12-15}) for oleic acid TAG.

The peaks at 25.9 and 25.5 ppm may also indicate a trans isomer, although it was not confirmed. Examination of the coupling constants in ^1H NMR may indicate if trans isomers were present.

The ratio of oxypropylene backbone to fatty acid methyl areas was 3.4:1, slightly lower than the ratio found for the day 0 sample (3.8:1), which may suggest a decrease in oxypropylene backbone component concentration relative to the fatty acid concentration. The ratio between the oxypropylene backbone methyls at 17.2 and 16.6 ppm was about 1.8:1, so that ratio stayed approximately the same as the day 0 EPG-08 oleate sample (1.9:1).

The ^1H NMR spectrum of the SFF M EPG-08 oleate sample (figure not shown) is similar to that of the day 0 EPG-08 oleate, except for the peaks at 5.6, 4.0, 3.7, and 1.8 ppm. The peaks at 3.7 and 1.8 ppm may be an indication of the presence of a small amount of tetrahydrofuran (THF) in the sample, although that conclusion is speculation based on the chemical shifts and the use of THF in sample preparation. The peaks at 5.6 and 4.0 ppm may be indicative of the presence of dimer.

The resonances for the ^{13}C spectrum of the fractionated dimer (SFF D) of day 2 (24 h) EPG-08 oleate are presented in Table 3. There is a carbonyl peak at 173.3 ppm with a shoulder, which was similar to the SFF M sample. Again, the olefinic carbons are not differentiated between the 1,3- and 2-glycerol carbons, as was expected (Gunstone, 1990b, 1991a; Wollenburg, 1990, 1991). The methine peak at 107.7 ppm and the methylene peaks at 67.9, 25.5, and 23.9 ppm were present in greater concentrations than for the SFF M or day 0 EPG-08 oleate samples. In sucrose polyesters (SPEs), a peak at 105−102 ppm was evident and assigned to the $C_{2'}$ of the sugar moiety bound to an oxygen through an ether bond (Ríos et al., 1994).

For the EPG-08 oleate SFF D sample (day 2), the oxypropylene backbone to fatty acid methyl carbon ration was 3.4:1, the same as for the SFF M EPG-08 oleate sample but less than for the day 0 sample (3.8:1). The oxypropylene backbone (17.2 ppm) to oxypropylene backbone (16.6 ppm) methyl carbon ratio was 1.8;1, again similar to those for the day 0 (1.9:1) and SFF M (1.8:1) samples.

The ^1H spectrum for the SFF D EPG-08 oleate (day 2) sample (figure not shown) contains peaks at 5.6, 4.0, 3.8, and 1.8 ppm. The resonance at 5.6 ppm may be an olefinic proton bound to a carbonyl, aldehyde, or carboxylic acid. The peak at 4.0 ppm may be on a methylene bound to a hydroxyl or a methine or methylene attached to an ether. The ratio of the olefinic (5.6−5.1 ppm) and divinylmethylene (3.0−2.6 ppm) protons to the aliphatic (2.5−0.6 ppm) protons was 1.0/14.42 = 0.069 or 6.9%. This value was lower than for the day 0 or SFF M EPG-08 oleate samples and indicates that oxidation had occurred (Saito and Udagawa, 1992).

Comparison of Olestra monomer and dimer (separated by preparative HPSEC) ^1H NMR spectra indicated that the only real difference was a broadening of the resonances of the dimer due to the increased viscosity of the dimer compared to the monomer, causing increased transverse relaxation times (T_2) (Gardner and Sanders, 1990). The 78−70 ppm region for all of the EPG-08 oleate samples was the same as for day 0, the fractionated monomer (SFF M), and the fractionated dimer (SFF D) samples, which may indicate that the oxypropylene was not modified during heating and/or involved in dimeric TAG formation.

For ^{13}C NMR spectrum of the unheated triolein sample, two peaks were present in the carbonyl region at 173.1 (1,3-) and 172.7 (2-) ppm (Table 4) (Gunstone, 1990a, 1993a; Wollenburg, 1991; Aursand et al., 1993; Medina et al., 1994). In palm oil, the 1,3- peak was smaller than the 2-carbonyl peak for oleic acid, different from that for triolein (Ng, 1985). The DEPT spectrum resonances have not been presented. There are two

carbonyl peaks for triolein rather than one major peak, as is the case for the day 0 EPG-08 oleate sample. Even though the fatty acid (oleic acid) was the same at each glycerol carbon, the C_1 1,3- and 2-positions could still be differentiated for triolein. The peaks at 129.904 (C_{10}), 129.6 (1,3- C_9), and 129.6 (2- C_9) ppm are for the olefinic carbons (Ng, 1984, 1985; Gunstone, 1990b, 1991a,b; Wollenburg, 1990, 1991; Husain et al., 1993).

Only two peaks were seen in the olefinic region of the day 0 and SFF M EPG-08 oleate samples (Table 1). In palm oil, the 1,3- peak was smaller than the 2-olefinic peak for oleic acid, different from that determined for triolein (Ng, 1985). Thus, in the regular TAG, the olefinic carbons at the C_9 position are differentiated, while the C_{10} carbons are not.

The resonance for the glycerol carbons for the triolein sample occurs at 68.8 ppm for the 2-glycerol carbon and at 62.0 ppm for the 1,3-glycerol carbons (Gunstone, 1990b; Wollenburg, 1991; Sacchi et al., 1993; Medina et al., 1994). These peaks were not present in the unheated day 0 and SFF M EPG-08 oleate (Table 2). The glycerol peaks in the "modified" TAG moved downfield compared to the glycerols in the regular TAG as evidenced by the lack of peaks in the 69−62 ppm range (Table 4).

The peaks at 34.1 and 33.9 ppm are indicative of the C_2 in the 1,3- and 2-positions, respectively, in glycerol (Table 4) (Gunstone, 1990a,b, 1991b, 1993a,b; Wollenburg, 1991; Aursand et al., 1993; Husain et al., 1993). The peak at 31.9 ppm was for the ω3 carbon.

The methylene envelope ($C_{4-7,12-15}$) was the group of peaks from 29.7 to 28.9 ppm (Table 4). The two peaks at 27.2 and 27.1 ppm are for the cis allylic carbons C_{11} and C_8, respectively. The two peaks at 24.8 (2-) and 24.8 (1,3-) ppm are for C_3. The peak at 22.6 ppm is for the ω2 carbon. The ω1 carbon peak is at 14.1 ppm (Gunstone, 1990a,b, 1991b, 1993a,b; Wollenburg, 1991; Aursand et al., 1993; Husain et al., 1993). There appeared to be no trans isomer present in the sample.

The ^{1}H NMR spectrum of the unheated triolein sample (figure not shown) contains two set of peaks from 5.37 to 5.24 ppm that are indicative of the olefinic protons and the 2-glycerol proton, while the peaks from 5.3 to 5.2 ppm indicate the 2-glycerol proton. The two sets of peaks from 4.3 to 4.26 and 4.15−4.10 ppm are for the 1,3-glycerol protons (Wollenburg, 1991; Aursand et al., 1993; Henderson et al., 1994). The protons on the methylene α to the carbonyl are the peaks from 2.32 to 2.27 ppm. The peaks from 2 to 1.97 ppm are the protons on the allylic carbons. The peaks at 1.59 are the protons on the methylene group β to the carbonyl. The methylene peaks are at 1.28 and 1.25 ppm, and the methyl peaks are at 0.88−0.85 ppm. The ratio of the olefinic (5.6−5.1 ppm) plus divinylmethylene (3.0−2.6 ppm) protons to the aliphatic (2.5−0.6 ppm) protons is 1.21/16.41 = 0.074 or 7.4%.

The resonances or peaks for the ^{13}C NMR and DEPT spectra for the SFF M day 4 (48 h) triolein are not shown. The purity (as determined by HPSEC) of the isolated SFF M fraction was 98.8%. The carbonyl peaks (C_1) are split into two at 173.2 (1,3-) and 172.8 (2-) ppm, with a shoulder on the α peak at 173.1 ppm. This is similar to unheated triolein spectrum. The C_{10} olefinic carbon is at 129.9 ppm, and the C_9 olefinic is at 129.6 (1,3-) ppm and 129.6 (2-) ppm (Ng, 1984, 1985; Gunstone, 1990a, 1991a,b, 1993a,b; Wollenburg, 1990, 1991; Husain et al., 1993; Medina et al., 1994). The methine peak at 107.8 ppm may have been due to the dimer fraction included in the SFF M. This peak was not present in the unheated sample.

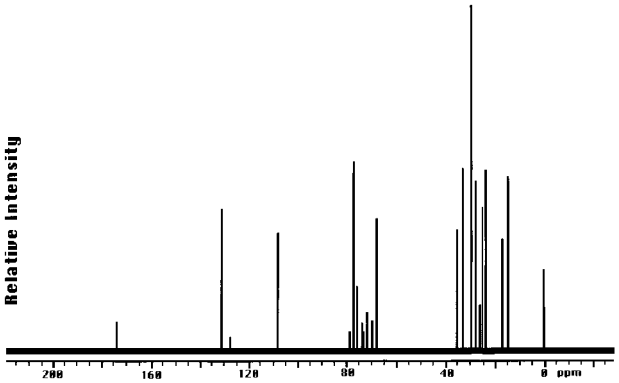

Figure 4. ^{13}C NMR spectrum of a SFF dimeric TAG isolate of heated sample of oleic acid esterified propoxylated glycerol (day 2) (EPG-08 oleate).

There are a number of peaks present in the glycerol carbon region that may indicate changes in the glycerol, oxypropylene backbone, and/or fatty acid environments or the presence of dimer in the sample. The methine peak at 69.7 ppm is the 2-glycerol carbon, and the methylene peak at 62.0 ppm is for the 1,3-glycerol carbons (Gunstone, 1990b; Wollenberg, 1991; Sacchi et al., 1993; Henderson et al., 1994). The peak at 68.2 ppm is a methine, and the peaks at 67.5 and 64.9 ppm are methylenes according to DEPT analysis (figure not shown). The methine at 68.2 ppm may be indicative of a change in the fatty acid environment for the 2-glycerol carbon or intradimerization within a TAG since this peak is present when the sample contains dimer and occurs on the inside glycerol carbon.

The peaks at 67.5 and 64.9 ppm are methylenes, which may be due to changes in the oxypropylene backbone or fatty acid environment due to interdimerization between TAGs. The peaks at 58.8 and 58.8 ppm were not identified. The peaks in the 35−0 ppm region are similar to the peaks in the unheated triolein sample. However, there is a trans isomer at 32.5 (C_8, C_{11}) (Gunstone, 1993a). There are peaks at 26.0 and 23.9 ppm, which were not identified and may be due to the presence of dimer.

The ^{1}H spectrum resonances for the triolein SSF M fraction (figure not shown) has peaks at 5.6, 3.9, 3.7, and 1.8 ppm that were not in the unheated triolein sample; otherwise, the spectra were very similar. These peaks may be indicative of the presence of a dimeric compound. The peaks at 5.6 ppm may be due to an olefinic hydrogen α to a carbonyl, aldehyde, or carboxylic acid. The peak at 3.9 ppm may be a methylene α to a hydroxyl or a methine or methylene α to an ether. The ratio of olefinic (5.6−5.1 ppm) plus divinylmethylene (3.0−2.6 ppm) protons to aliphatic (2.5−0.6 ppm) protons is 1.05/15.31 = 0.069 or 6.9%. This is lower than the 7.4% for the unheated triolein sample.

The ^{13}C NMR (Figure 4) and DEPT spectra (figures not shown) resonances for the SFF D day 2 (24 h) triolein sample have carbonyl peaks at 173.3, 173.2, 173.2, and 172.8 ppm that are indicative of dissimilar environments due to possible dimerization between or within fatty acids. The olefinic peak for C_{10} was at 129.9 ppm and for C_9 was at 129.6 (1,3-) and 129.6 (2) ppm (Ng, 1984; Gunstone, 1990b, 1991a,b; Wollenburg, 1990, 1991; Husain et al., 1993). The peak at 127.5 ppm may have been an instrument spike. The three methine peaks at 107.9, 107.5, and 106.3 ppm may be indicative

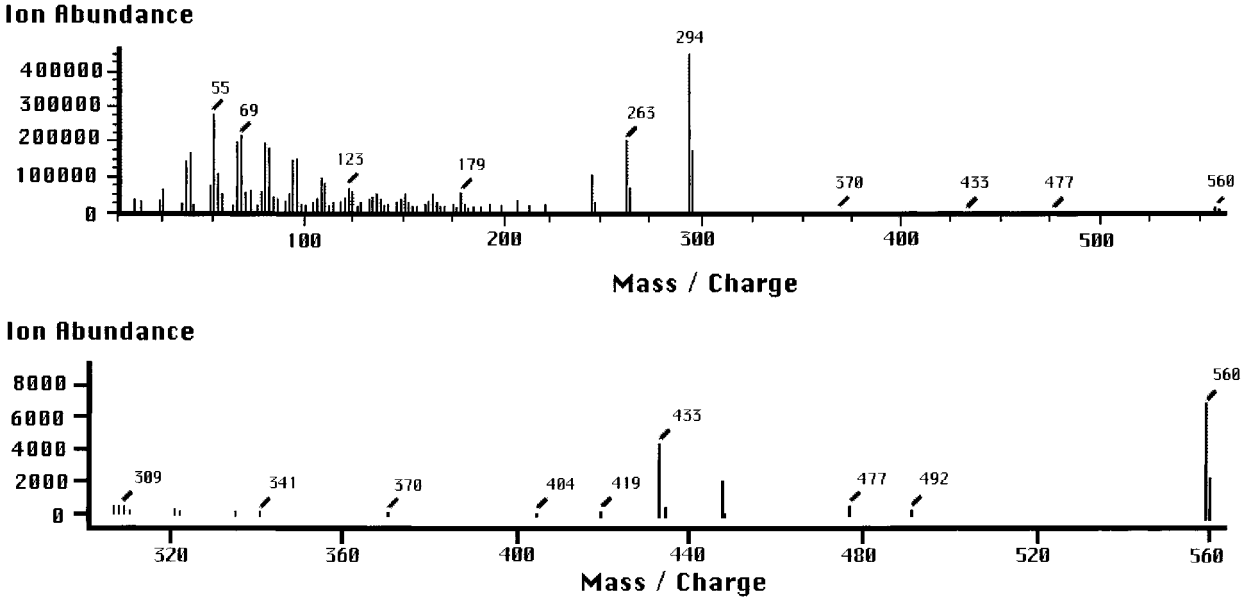

Figure 5. Total ion chromatogram for the dimeric FAMEs from the SFF D triolein fraction.

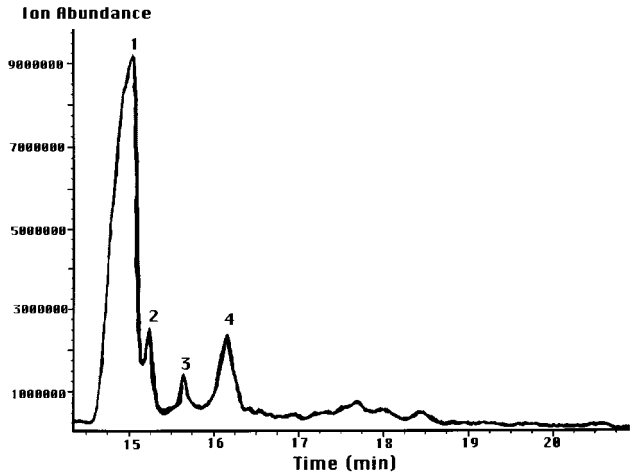

Figure 6. EI mass spectrum from peak 1 of the dimeric FMAEs from the SFF D triolein fraction.

of double-bond formation near the carbonyl, as the chemical shift between two olefinic carbons becomes larger and is shifted upfield when the double-bond position is closer to the carbonyl (Ng and Koh, 1988).

In the glycerol region, methylene peaks are at 67.6, 67.6, 67.4, and 62.0 ppm. The methine peak is at 68.8 ppm and is from the 2-glycerol carbon (Gunstone, 1990b; Wollenburg, 1991; Sacchi et al., 1993; Henderson et al., 1994). The environment for this carbon has not changed significantly. The peak at 62.0 ppm is from the 1,3-glycerol carbons, while the other methylene peaks may be due to unsaturation closer to the carbonyl, a change in the oxypropylene backbone or fatty acid environment, or dimerization between TAGs. The peaks have shifted downfield from the original 1,3-glycerol carbons.

The spectrum from 35 to 0 ppm is similar to that for the SFF M triolein sample, except for the methylene peaks at 23.9, 23.8, and 23.7 ppm. These peaks, along with the additional peaks in the glycerol region and the peaks upfield from the olefinic region, are characteristic of the SFF D samples and may indicate that dimerization is occurring between the olefinic and carbonyl bond regions of fatty acids. These peaks are also present in the unheated oil (EPG-08 oleate), indicating that the oxypropylene backbone is not necessary for these peaks to appear. In summary, the oxypropylene backbone does not appear to be involved in any of these reactions.

The ^{1}H NMR spectra peaks at 5.8−5.6, 4.0−3.9, and 1.9−1.8 ppm are not in the day 0 or SFF M triolein sample (figures not shown). The peaks at 5.8−5.6 ppm may be indicative of C═C near a carboxylic acid, carbonyl, or aldehyde functional groups or attached at the end of a chain (−CH_2) or an alcohol. The peaks at 4.0−3.9 ppm may be indicative of an ether bond, a double bond near an ether bond, a double bond attached to a methylene attached to an ether, or an alcohol. The peaks at 1.9−1.8 ppm may indicate a methyl attached to a double bond, a methyl attached to a carbonyl, a methylene, or an alcohol. The ratio of olefinic (5.6−5.1 ppm) plus divinylmethylene (3.0−2.6 ppm) to aliphatic (2.5−0.6 ppm) protons was 0.98/16.63 = 0.059 or 5.9%. This value is less than the 7.4 and 6.9% for day 0 and SFF M, respectively. This decrease also occurs during fish oil oxidative deterioration (Saito and Udagawa, 1992).

For each of the SFF D samples there were common peaks at approximately 107.7, 67.7, and 23−25 ppm in the ^{13}C NMR spectrum (figures not shown). Common peaks in the ^{1}H NMR spectrum were also evident at approximately 5.6, 3.9, and 1.8 ppm.

A 2-D HETCOR (^{1}H,^{13}C) NMR spectrum was also obtained to correlate carbons with protons (figure not shown) and to facilitate peak assignments. The SFF D triolein fraction was used because it had the largest number of peaks in all three regions (olefinic, glycerol, and aliphatic). The methine peaks at 107.9, 107.5, and 106.3 ppm correspond to the olefinic proton peak at 5.58 ppm. These olefinic carbons are upfield from the C_{10} and C_9 olefinic carbons (129 ppm). However, the olefinic proton peak at 5.58 for the upfield methines is downfield from the olefinic proton peak for the C_{10} and C_9 (5.32 ppm). Ng and Koh (1988) found that as the double bond moved closer to the carbonyl, the chemical shift between olefinic carbons became larger.

The glycerol peaks at 68.792 and 62.048 ppm corresponded to the 1,3- and 2-positions, respectively. The methine glycerol peak at 68.79 ppm (2-) corresponded to the peak at 5.25 ppm on the ^{1}H spectrum, as expected (Gunstone, 1990b; Wollenburg, 1991; Sacchi et al., 1993; Henderson et al., 1994). The methylene glycerol carbon

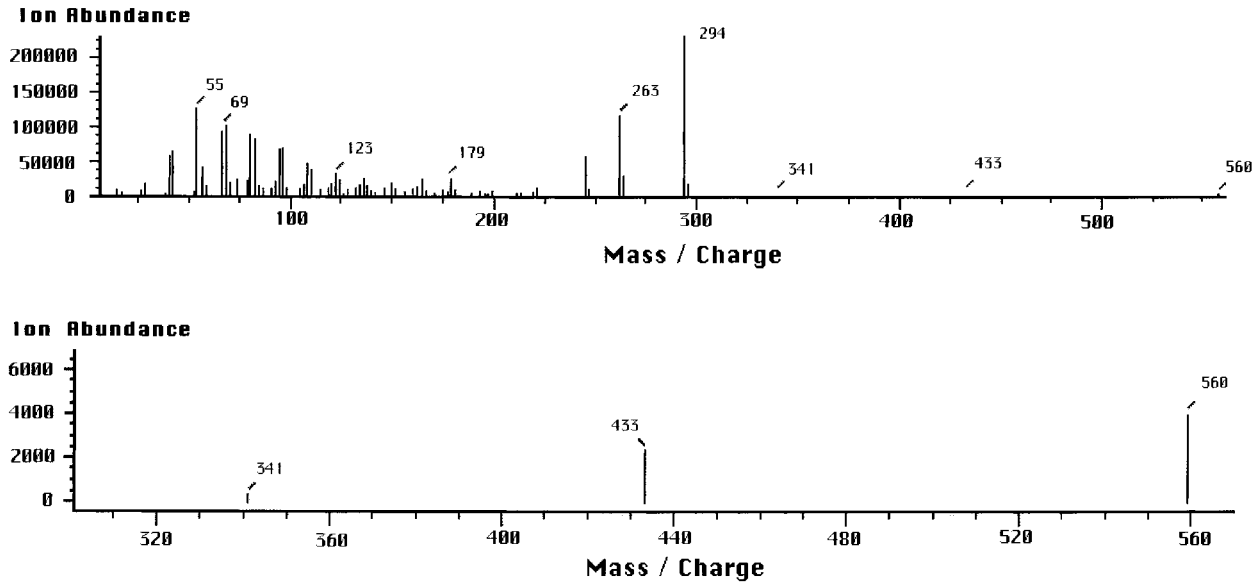

Figure 7. Total ion chromatogram for the dimeric FAMEs from the SFF D EPG-08 oleate fraction.

peaks at 62.0 ppm (1,3-) corresponded to the two peaks at 4.26 and 4.13 ppm in the ^{1}H spectrum, as would be expected. The methylene peaks at 67.64, 67.59, and 67.37 ppm correspond to the peak at 3.96 ppm in the ^{1}H spectrum. These methylene peaks are downfield from the 1,3-glycerol carbons. The peak at 3.96 ppm corresponds to a methylene α to an ether or alcohol. The assignments for peaks at 23.88 and 23.76 ppm are not clear.

Gas Chromatography (GC)/Mass Spectrometry (MS) Analyses. Figure 5 is a total ion chromatograph (TIC) of the dimeric FAMEs from the transesterified dimeric (SFF D) triolein fraction eluting from 15 to 16.2 min. After transesterification, a dimeric FAME standard (Empol 1008, Henkel, Emery Group, Cincinnati, OH), composed of a variety of isomers, eluted from 13 to 21 min. Comparison of retention times (T_r) of the standard to the retention times of the component peaks in Figure 5 suggests the peaks eluting at 15−16.2 min were probably dimeric FAMEs. The mass spectrum for peak 1 is shown in Figure 6. The mass spectra from the three other peaks are not presented. No molecular ion (M^*) was present in any of the methyl oleate (MeOL) dimeric FAME mass spectra. The fragmentation pattern indicated that peak 1 was a dehydro dimer of MeOL (Christopoulou and Perkins, 1989d). The GC peak profile was similar to peaks found for the dehydro dimer of MeOL (Christopoulou and Perkins, 1989c). The peaks at *m/e* 41−43, 55, 67−69, 81−83, 95−97, 109−111, 123−125, 137−139, 151−152, 165−167, and 179 were indicative of a long-chain alkene series. The peaks at *m/e* 295 and 294 are (M/2) − 1 and M/2, while the peaks at 264 and 263 are M/2 − CH_3O and M/2 − CH_3-OH peaks. The peaks at 561 and 560 are M − CH_3O and M − CH_3OH peaks. The parent ion, M (*m/e* 592), was not present. The peaks at 492 and 477 were for M − $(CH_2)_6CH_3$ and M − $(CH_2)_7CH_3$, respectively. The peaks at *m/e* 447, 433, 419, and 404 could be due to the fragments M − $(CH_2)_6CO_2CH_3$, M − $(CH_2)_7CO_2CH_3$, M − $(CH_2)_8CO_2CH_3$, and M − $(CH_2)_9CO_2CH_3$, respectively.

The fragmentation pattern for peak 2 (figure not shown) was very similar to that for peak 1. However, there were much greater abundances for peak 2 at an *m/e* of 307−311, 323−321, and 338. The *m/e* peaks at 309 and 311 may possibly indicate the presence of oxygen (Gardner and Sanders, 1990; Christopoulou and Perkins, 1989d). *m/e* peaks at 293 and 295 plus oxygen would be *m/e* peaks of 309 and 311. However, there was no M peak over 600 as one would expect for an oxygenated dehydro dimeric FAME (Gardner and Sanders, 1990; Christopoulou and Perkins, 1989b,d). Other oxygenated dimeric FAMEs that have been identified by previous investigators include a hydroxylated dimer, an ether bridged dimer, and a keto dimer. Chemical ionization (CI) is a softer ionization technique that results in less fragmentation than EI and may contain a parent ion for the dimeric FAME.

Infrared (IR) analysis indicated a small percentage of free hydroxyl groups may be present as evidenced by a small peak at ≈3400 cm^{-1} (figure not shown). This small peak was also present in the unheated triolein sample. The exact percentage of free OH groups present in the dimeric FAME sample is unknown. A couple drops of dinitrophenylhydrazine in 95% ethanol were added to a triolein SFF D dimeric FAME sample to check for the presence of carbonyls. A slightly cloudy solution developed, indicating the possibility that a small amount of carbonyl may be present. No carbonyl precipitated out of solution as one would expect if a substantial amount of carbonyl functional groups were present. There were no other dimeric FAMEs of MeOL identified (Christopoulou and Perkins, 1989b,d). Chang et al. (1978) isolated a noncyclic carbonyl dimeric FAME, after derivatization, from heated triolein. MeOL dimeric FAMEs are formed from free radical reactions and not from the Diels−Alder reaction (Jensen and Møller, 1986).

The peaks at *m/e* of 294 for peak 3 were much lower in relative abundance than peaks 1 and 2 compared to the other peaks (figure not shown). Peaks at *m/e* 419, 403, and 335 were present in greater relative abundances than in peaks 1 and 2. The peaks at *m/e* 309 and 311 were not present at very large concentrations for peak 1 or 3. Peak 3 does not appear to be oxygenated and may be an isomer as suggested by the low abundance of *m/e* 309 and 311.

Peak 4 (Figure 7) has an *m/e* at 543 which may be M − CH_3OH − H_2O and may indicate the presence of a hydroxy group. The peaks at *m/e* 324−326 are unique to peak 4 and could possibly indicate an ether linkage in the FAME. Although that has not been reported in the deep fat frying literature for MeOL, it has been

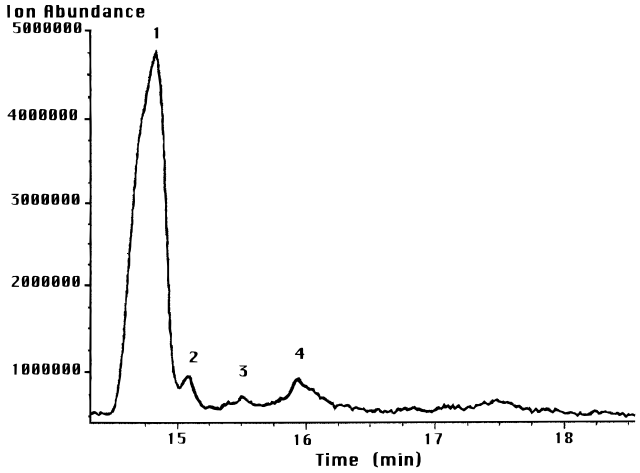

Figure 8. EI mass spectrum from peak 1 of the dimeric FAMEs from the SFF D EPG-08 oleate fraction.

suggested as a possible dimer product for methyl linoleate (MeLN) dimeric FAMEs (Garssen et al., 1972; Gardner and Sanders, 1990). Peak 1 (Figure 8) from EPG-08 oleate SFF D had a fragmentation pattern that was similar to that of peak 1 for the SFF D triolein sample (Figure 6), suggesting methyl oleate. Peaks 2−4 of the SFF D EPG-08 oleate fraction were not present in sufficient amount to obtain a mass spectra.

CONCLUSION

An analytical fractionation technique using supercritical carbon dioxide (SC CO_2) was successfully developed for obtaining relatively pure monomeric and dimeric TAG fractions of heated EPG-08 oleate and triolein for further structural analysis.

Carbon-13 and DEPT NMR analysis indicated that the oxypropylene backbone and glycerol backbone of EPG-08 oleate was not altered significantly, as was the fatty acid component of the molecule. Three groups of peaks specific for the fractionated dimer samples were seen at approximately 107, 67−68, and 23 ppm. The peak at 107 ppm indicates a double bond that may have migrated toward the carbonyl portion of the FA chain, as indicated by the large chemical shift between the olefinics at 129 ppm and the peak at 107 ppm (Ng and Koh, 1988). The peaks at 67−68 ppm were methylene carbons and may indicate that the chemical environment for the α glycerol carbon changed during dimerization. The peak at 23 ppm suggests that a quaternary carbon between C_2 and C_{17} in the fatty acids is involved in the dimerization process. From the NMR results, it appears that dimerization probably occurs in the fatty acid portion of the backbone rather than the oxypropylene backbone portion.

GC/MS analysis indicated the formation of dimeric FAMEs from heated TAGs containing oleic acid. While this does not prove that the oxypropylene backbone component is not involved in dimeric TAG formation, it does indicate that the fatty acid portion of the chain is involved in dimer formation.

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Received for review July 16, 1997. Revised manuscript received October 14, 1997. Accepted October 15, 1997.[⊗] Support was provided by ARCO Chemical Co. (Newtown Square, PA), Best Foods, a Division of CPC International, Inc. (Englewood Cliffs, NJ), the University of Illinois College of Agricultural, Consumer and Environmental Sciences, the University of Illinois Agricultural Experiment Station, and the University of Illinois Research Board.

JF970614W

[⊗] Abstract published in *Advance ACS Abstracts*, November 15, 1997.