Characterization of Triacylglycerols in Saturated Lipid Mixtures with Application to SALATRIM 23CA

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SALATRIM 23CA is a member of the SALATRIM family of triacylglycerols developed by RJR Nabisco Co. It is an interesterification product of triacetin, tripropionin, and hydrogenated canola oil. To characterize this sample, a combination of reversed-phase high-performance liquid chromatography and high-temperature capillary gas chromatography (HTCGC) in sequence was used to separate the SALATRIM 23CA into individual components. An on-line coupling of HTCGC with positive chemical ionization mass spectrometry revealed the structure of the individual components. To obtain detailed quantitative information, a combination of a 5-m OV-1 capillary column and a 25-m methyl 65% phenyl silicone Al-clad column was used to determine the quantities of acylglycerols. A total of 55 components were characterized in this study. They included 52 triacylglycerols and 3 diacylglycerols.

INTRODUCTION

SALATRIM 23CA is an interesterification product prepared from the reaction of 11:1:1 mole ratios of triacetin, tripropionin, and hydrogenated canola oil (Klemann et al., 1994). Like other complex lipid mixtures, it is difficult to separate and detect all of the components by using a single chromatographic method.

The combination of two or more chromatographic methods in sequence is widely used for lipid characterization. For example, Ratnayake et al. (1989) identified several novel branched-chain fatty acids by fractionation of red fish oil triacylglycerols using silver ion thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GCMS) analysis. Tekeuchi and Ackman (1987) separated rock crab triacylglycerols by reversed-phase high-performance liquid chromatograph (HPLC) and then determined their carbon numbers by GC. More recently, Laaksa and Christie (1991) used silver ion chromatography and reversed-phase HPLC in sequence to obtain information on the composition of herring triacylglycerols.

Gas–liquid chromatography (GLC) is the most valuable and efficient choice for lipid separation and quantitations (Kuksis and Myher, 1987). High-temperature gas chromatography (HTGC) using short packed columns has been long known to provide quantitative profiles of fats and oils (IUPAC, 1985). The quantitation of natural triacylglycerols by capillary GC was first published by Monseigny et al. (1979). Later, Mares and Husek (1985) published a detailed study of quantitative GLC of triacylglycerols on a short fused silica capillary column with a chemically bonded nonpolar stationary phase. Quantitative capillary GC of triacylglycerols using a methyl 65% phenyl silicone capillary column with FID has been published by Hinshaw and Seferovic (1986) and by Gilkison (1989). The column provides excellent resolution of triacylglycerols. However, the low recovery of high molecular weight and highly unsaturated triacylglycerols has limited its use for quantitative work. In the field of lipid structure identification, intact lipids have been identified by on-line liquid chromatography–mass spectrometry (LCMS) with direct inlet technique (Kuksis et al., 1991), moving-belt interface (Jungalwala et al., 1984), thermospray interface (Kim and Salem, 1986), and particle beam interface (Huang et al., 1994). The on-line coupling of GCMS has also been applied in the identification of triacylglycerols with a packed column GC (Murata, 1977) or with a capillary column GC (Oshima et al., 1988).

This paper deals with the use of HPLC, HTCGC, and positive chemical ionization mass spectrometry (PCI-MS) methods in the fractionation, separation, identification, and quantification of the individual acylglycerol components of SALATRIM 23CA. These techniques provide a general approach for the characterization of triacylglycerols in natural and modified saturated lipid mixtures.

EXPERIMENTAL PROCEDURES

The chemical characterization of triacylglycerols in SALATRIM 23CA was performed according to the steps outlined in Figure 1.

Material. SALATRIM 23CA was obtained from EPL Bio-Analytical Services, Inc., Decatur, IL. The sample was stored in an amber glass bottle with a Teflon-lined screw cap, flushed with nitrogen, and stored at −20 °C until used. Tricaproin, triheptanoin, tricaprylin, trinonanoin, tricaprin, triundecanoin, trilaurin, tritridecanoin, trimyristin, tripentadecanoin, tripalmitin, triheptadecanoin, tristearin, and trinonadecanoin standards were purchased from Nu Check Prep, Inc., Elysian, MN.

Synthesis of Diacetylstearoylglycerol and Distearoylacetylglycerol. Diacetylstearoylglycerol was prepared by adding 2 g of stearoylglycerol (1-monostearin, 99%, Sigma Chemical Co., St. Louis, MO) to 1.2 g of acetic anhydride (98%, Aldrich Chemical Co., Milwaukee, WI) in a 25-mL round-bottom flask equipped with magnetic stir bar and reflux condenser. The system was heated to 136–138 °C for 8 h at atmospheric pressure. The crude triacylglycerol product was dissolved in 50 mL of chloroform followed by neutralization with 50 mL of 3 M sodium bicarbonate. The solution was extracted with chloroform (5 × 50 mL), and the organic layers were combined, dried over magnesium sulfate, filtered, and evaporated under vacuum. A total of 1.76 g of product was obtained.

Distearoylglycerol (1,3-distearin) was synthesized according to the methods published by Lok (1980) and Lok et al. (1985). Distearoylacetylglycerol was prepared from 1124.4 g of distearoylglycerol added to 192.9 g of acetic anhydride in a 2-L, three-neck round-bottom flask equipped with reflux condenser. The system was heated to 145 °C for 8 h at atmospheric pressure. The reaction flask was then equipped with a vacuum distillation head and receiver. The acetic acid side product and other volatile components were removed in three stages: vacuum distillation

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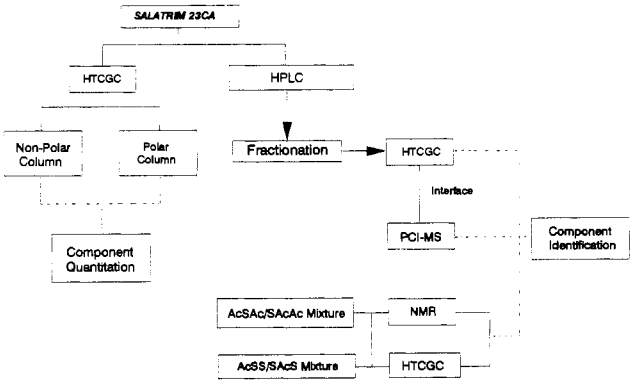

Figure 1. Chemical characterization of SALATRIM 23CA.

at 100 mmHg and 85 °C, followed by falling-film distillation with mesitylene as solvent (168 °C, <1 mmHg), and then steam deodorizing in a 2-L laboratory-scale deodorizer at 210 °C (<1 mmHg) for 90 min with 50 mL of distilled water. A total of 1049.3 g of product was obtained.

Both product mixtures were characterized by ^{13}C nuclear magnetic resonance (NMR) using the methods discussed by Henderson et al. (1994) and by HTCGC using a methyl 65% phenyl silicone column, as described below. The results of the NMR analyses of diacetylstearoylglycerol and distearoylacetylglycerol were used to determine the elution order of positional isomers in the HTCGC chromatogram. There are other milder synthetic techniques than the ones we used to obtained the above material. However, the subject is beyond the scope of this paper.

Fatty Acids Determination. SALATRIM 23CA was analyzed for its fatty acid components by gas chromatography of the fatty acid methyl esters (FAME). The transesterification of SALATRIM 23CA triacylglycerols into their respective methyl esters was performed using a 0.2 N methanolic solution of *m*-(trifluoromethyl)phenyl trimethylammonium hydroxide (Meth-Prep II solution obtained from Alltech Associates, Deerfield, IL) according to the procedure described by McCreary et al. (1978). The gas chromatographic analysis of FAME was performed using a Hewlett-Packard Model 5890 Series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector. A Supelco SP2340 capillary column (60 m × 0.25 mm i.d. with 0.2-μm film thickness obtained from Supelco Inc., Supleco Park, Bellefonte, PA) was used for the separation. The injector and detector temperatures were both set at 245 °C. The initial oven temperature was set at 60 °C for 7 min followed by ramping from 60 to 225 °C at 8 °C/min and then held at 225 °C for 15 min. The helium flow rate was set at 20 cm/s. A mixture of standard methyl esters, containing equal weights of methyl acetate, methyl propionate, methyl butyrate, methyl caproate, methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, methyl arachidate, methyl behenate, and methyl lignocerate, was used for comparison. All methyl ester standards were obtained from Alltech Associates.

Fractionation of SALATRIM 23CA by Reversed-Phase HPLC. SALATRIM 23CA was fractionated into eight broad fractions with a Waters 815 HPLC system equipped with an autoinjector, UV absorbance detector, fraction collector, and a Waters Baseline data system (Waters Associates, Milford, MA). All solvents used in the HPLC experiments were Baker Analyzed HPLC grade (J. T. Baker Chemical Co., Phillipsburg, NJ). Two Supelcosil LC-18 stainless columns (15 cm × 4.6 mm i.d. with 5-μm particle size) in series were used for the fractionation. The columns were obtained from the Supelco. The mobile phase was run at 1 mL/min with 100% acetonitrile for the first 30 min followed by a step gradient to a 75/25 v/v mixture of acetonitrile/*tert*-butyl methyl ether for another 30 min. The UV detector was operated at 220 nm using 0.25 AUFS. A 5% (w/v) solution of SALATRIM 23CA was prepared in a 50/50 (v/v) mixture of acetone and tetrahydrofuran (THF), 20 μL of which was used for each injection. A total of 110 injections were made with the eluent from each injection collected in seven fractions, hereafter referred to as fractions Fr-A through Fr-G. An eighth fraction (Fr-H) was collected following the 110th injection, obtained by flushing the column with 50 mL of THF. Solvent was removed from the pooled fractions, and the remaining residue from each fraction was weighed.

Analysis of SALATRIM 23CA Samples by the HTCGC with a Quadrex Methyl 65% Phenyl Silicone Capillary Column. Analysis of triacylglycerol standards and SALATRIM 23CA was performed on a Hewlett-Packard 5890 Series II GC. A Quadrex aluminum-clad column (25 m × 0.25 mm i.d.) with a 0.1-μm film of methyl 65% phenyl silicone column was used for the separation. A deactivated Al-clad precolumn (0.5 m × 0.53 mm i.d.) was coupled to the analytical column via a butt connector (Quadrex Corp., New Haven, CT). The oven temperature was ramped from 100 to 200 °C at 30 °C/min, followed by ramping from 200 to 360 °C at 6 °C/min, and then held at 360 °C for 15 min. On-column injection was used with the injector operated in the temperature track mode, which keeps the injector temperature close to the oven temperature. Hydrogen was used as the carrier gas with a constant flow rate operating at 8.0 psi, 100 °C. The FID was operated at 380 °C. A 1000 ppm solution of SALATRIM 23CA was prepared in isooctane/chloroform (80/20 v/v) with 25 ppm of trilaurin added as an internal standard. This solution was injected in a 1-μL aliquot. Both isooctane and chloroform were obtained from Sigma.

Analysis of SALATRIM 23CA by HTCGC with Chrompack SIM-DIST CB Capillary Column. Analysis of triacylglycerol standards and SALATRIM 23CA according to their carbon number was performed on a Hewlett-Packard 5890 Series II GC. The separation was accomplished with a Chrompack SIM-DIST CB fused silica column (5 m × 0.32 mm i.d., 0.1-μm film thickness, Chrompack Inc., Raritan, NJ). A deactivated fused silica precolumn (0.5 m × 0.53 mm i.d.) was coupled to the analytical column via a butt connector (Quadrex). The oven temperature was ramped from 140 to 350 °C at 15 °C/min and held at 350 °C for 2 min. On-column injection was used with the injector operated in the temperature track mode. Hydrogen was used as the carrier gas with a constant flow rate operating at 5.5 psi, 140 °C. The FID was operated at 375 °C. A 2000 ppm solution of SALATRIM 23CA was prepared using undecane/toluene (95/5 v/v) with 50 ppm of trilaurin added as an internal standard. A 1-μL injection volume was used. Both undecane and toluene were obtained from the Sigma.

Identification of SALATRIM 23CA Components by High-Temperature Capillary Gas Chromatography with Positive Chemical Ionization Mass Spectrometry (HTCGC–PCI-MS). A Hewlett-Packard 5890 Series II GC coupled with Hewlett-Packard 5889 mass spectrometer was used for the analysis. The Hewlett-Packard 5890 GC was equipped with a pressure programmable on-column injector. A Quadrex methyl 65% phenyl Al-clad column (25 m × 0.25 mm i.d.) was used for separation. The GC temperature was programmed from 150 to 360 °C at 10 °C/min. The interface between the GC and mass spectrometer was maintained at 320 °C. The source temperature was set at 250 °C. Ammonia was used as the reagent gas at a source pressure of 1.0 Torr. The mass to charge ratios (m/z) of the various peaks were determined by the mass marker which had been calibrated with perfluorotributylamine (PFTBA; Hewlett-Packard). A scan range of m/z 250–1000 was used. A Hewlett-Packard UNIX ChemStation software package was used for data acquisition and data processing.

Precision Determination of the SALATRIM 23CA HTCGC Analysis. The precision of the SALATRIM 23CA analysis was established by quadruplicate injections under the HTCGC conditions outlined previously. The retention times were recorded, and the area percent of each peak was used for the precision determination. The standard deviations and coefficient of variation (CV) were computed according to the AOAC guide "Use of Statistics to Develop and Evaluate Analytical Method" (Wernimont, 1985).

Determination of the Correction Factors of Standard Triacylglycerols and Calculation of the Triacylglycerol Groups Weight Percentage in SALATRIM 23CA Using the Chrompack SIM-DIST CB Column. The determination of triacylglycerol correction factors and the calculation of triacylglycerol groups in SALATRIM 23CA followed the procedures

outlined in the IUPAC (1985) standardized method. Fourteen triacylglycerol standards were used. They were tricaproin, triheptanoin, tricaprylin, trinonanoin, tricaprin, triundecanoin, trilaurin, tritridecanoin, trimyristin, tripentadecanoin, tripalmitin, triheptadecanoin, tristearin, and trinonadecanoin. The correction factor (F_i) for each of the triacylglycerol standards was determined by the equation

$$F_i = (C_{si}/C_L)(A_L/A_{si})$$

where A_L is the peak area for trilaurin, A_{si} is the peak area for the ith standard triacylglycerol, C_L is the concentration (μg/mL) of trilaurin, and C_{si} is the concentration (μg/mL) of the ith standard triacylglycerol. A standard curve was generated from the standards using correction factor values versus the corresponding carbon number. The standard curve was used to calculate the correction factor of the components in SALATRIM 23CA.

The weight percent (W_{TGi}) of each group of triacylglycerols having the same carbon number as a percent of total triacylglycerol groups was calculated as

$$W_{TGi} = A_{TGi}/A_T \times 100\%$$

where A_{TGi} is the corrected peak area of triacylglycerol group i and A_T is the total corrected peak area of triacylglycerol groups in SALATRIM 23CA ($A_T = \sum A_{TGi}$).

The estimated recovery rate (R) of SALATRIM 23CA material in HTCGC analysis was calculated from the known weight percent of added internal standard (trilaurin) and its calculated weight percent following the equation

$$R = W_{si}/(W_{si} + W_t)(A_T/A_{TGsi})/A_{TGsi}$$

where W_{si} is the weight of trilaurin added, W_t is the weight of SALATRIM 23CA, and A_{TGsi} is the corrected peak area of trilaurin.

The corrected weight percent (CW_{TGi}) of each group of triacylglycerols having the same carbon number as a percent of SALATRIM 23CA is as follows:

$$CW_{TGi} = W_{TGi}R$$

Weight Percent of Individual Components Determination by Methyl 65% Phenyl Silicone GC Column Analysis. The weight percent of individual Salatrim 23CA components, WT_i, resolved on the methyl 65% phenyl silicone column is given as

$$WT_i = CW_{TGi} \times AR_i/ART_i$$

where CW_{TGi} is the corrected weight percent of all species with the same carbon number group obtained from the work with the SIM-DIST CB column analysis described above, AR_i is the peak area percent of the ith peak resolved with the methyl 65% phenyl silicone column, and ART_i is the sum of all the peak areas for individual components having the same carbon number ($ART_i = \sum AR_i$).

RESULTS AND DISCUSSION

Fatty Acids Determination. SALATRIM 23CA is an interesterification product of triacetin, tripropionin, and hydrogenated canola oil at a molar ratio of 11:1:1. It was found to have the following fatty acids: acetic (C2:0) acid, propionic (C3:0) acid, palmitic (C16:0) acid, stearic (C18:0) acid, oleic (C18:1) acid, arachidic (C20:0) acid, behenic (C22:0) acid, and lignoceric (C24:0) acid. Since SALATRIM 23CA contains short-chain acetic and propionic acids, the quantitative recovery of methyl esters from the reaction medium is difficult because of their high volatility and partial solubility in water (Iverson and Sheppard, 1977). The fatty acid data were used to assist in the identification of acylglycerols in mass spectra only.

Reversed-Phase HPLC Fractionation and HTCGC Separation of SALATRIM 23CA. GLC and reversed-phase HPLC are two of the best tools for triacylglycerol molecular species separation (Kuksis and Myher, 1987). Reversed-phase HPLC separates triacylglycerols according to the combination of fatty acyl residues and the total number of double bonds. Consequently, there is little or no resolution of positional isomers and species having the same equivalent carbon numbers (ECN) in spite of the differences in chain lengths, numbers of double bonds, and geometrical configuration (El-Hamdy and Perkins, 1981).

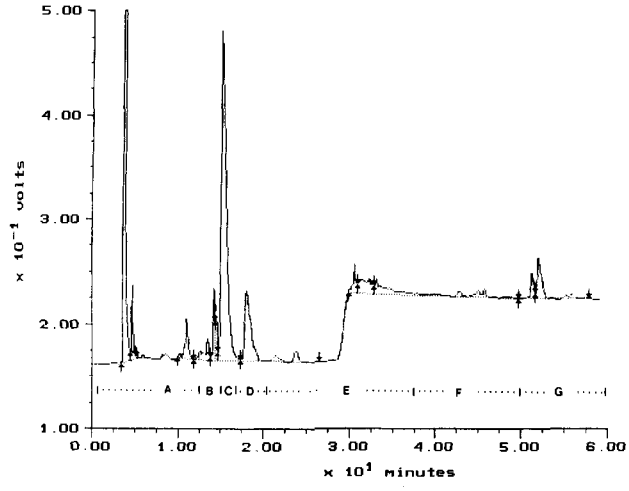

Figure 2. Reversed-phase HPLC fractionation of SALATRIM 23CA. The HPLC conditions are outlined under Experimental Procedures. The beginning and end of each fraction are indicated in the figure.

On the other hand, HTCGC separates triacylglycerols according to their respective vapor pressure at elevated temperature (Kuksis et al., 1984). Further resolution of the individual triacylglycerols by degree of unsaturation within the same carbon number (CN) can be accomplished by using a polar-phase methyl phenyl silicone column. Generally, the resolution of triacylglycerols obtained by capillary GC on a polar column is superior to that of HPLC or capillary SFC (Hinshaw and Seferovic, 1986). However, the sample loading capicity of a high-resolution capillary column is much less than that of a typical HPLC column. Minor triacylglycerols, especially those with higher molecular weights and high unsaturation, may not be detected without pre-enrichment. Therefore, reversed-phase HPLC was chosen for fractionation, and HTCGC with a methyl 65% phenyl silicone capillary column was selected for the final separation because of its high resolving power.

SALATRIM 23CA was fractionated into eight broad fractions by reversed-phase HPLC. The complete fractionation of SALATRIM 23CA is shown in Figure 2. Material collected from the HPLC fractionation accounts for about 95.8% by weight of the SALATRIM 23CA injected. HTCGC analysis of unfractionated and fractionated SALATRIM 23CA showed that some components appear in two or more HPLC fractions. This indicates that either some components were not well separated by reversed-phase HPLC or there is incomplete elution following each of the 110 injections. HTCGC profiles of HPLC fractions revealed some minor components that were not detected by HTCGC analysis of unfractionated SALATRIM 23CA. These minor components were enriched by repeated collections of HPLC fractions. A typical HTCGC profile of SALATRIM 23CA is shown in Figure 3.

Identification of SALATRIM 23CA Acylglycerols by HTCGC–PCI-MS. The identification of triacylglycerols by GC coupled with PCI-MS with ammonia as the reagent gas was first demonstrated by Murata and Takahashi (1977). In the positive [NH_3]CIMS experiments a triacylglycerol with molecular weight M produces

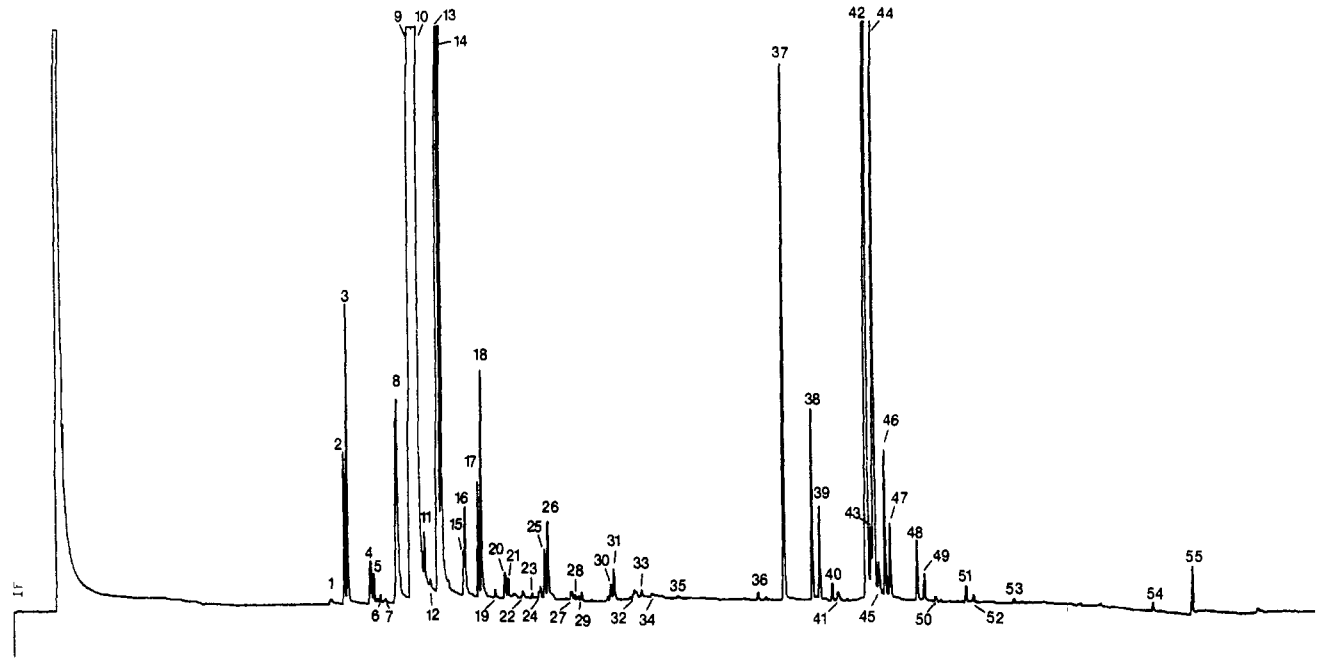

Figure 3. HTCGC (Quadrex methyl 65% phenyl silicone Al-clad column) analysis of SALATRIM 23CA. The GC conditions are discussed under Experimental Procedures. Peak identifications are shown in Table 2.

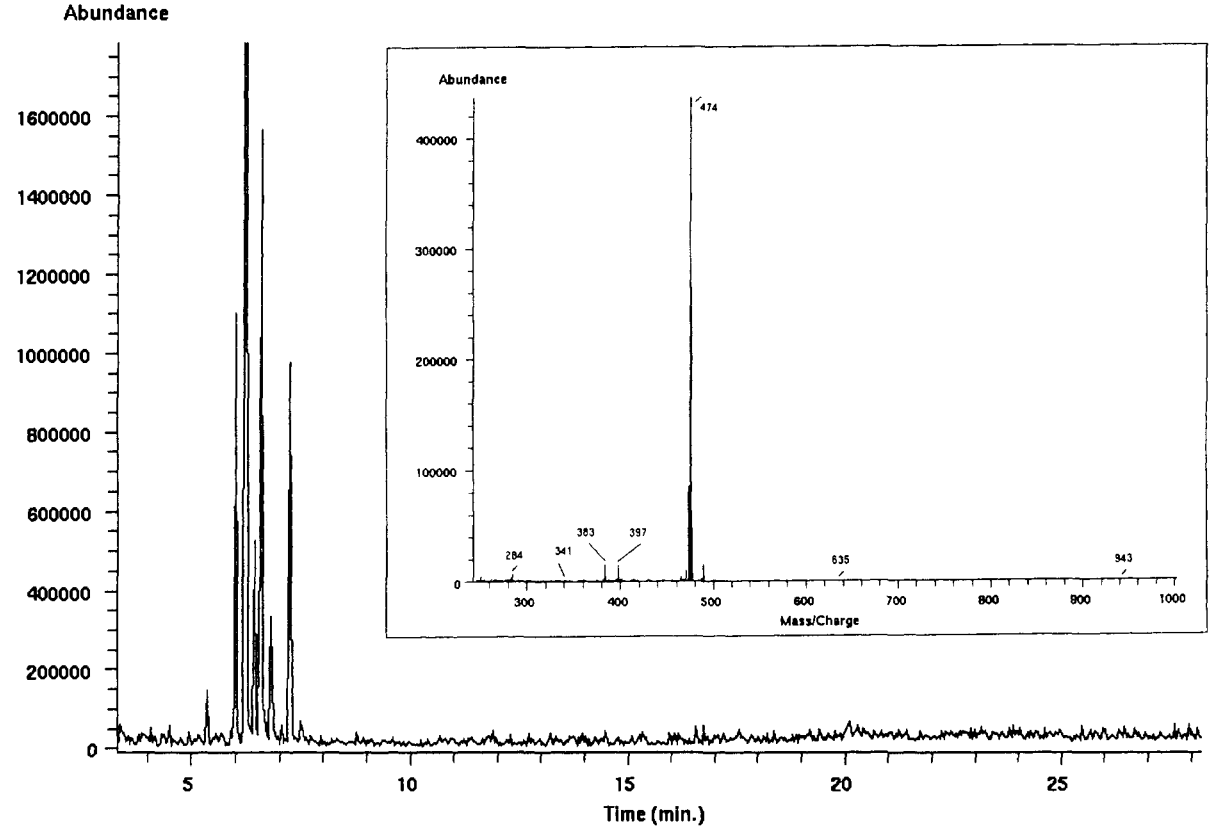

Figure 4. Positive chemical ionization total ion chromatogram of HPLC fraction E and positive chemical ionization spectrum of 2-stearoylacetylpropanylglycerol (at retention time 6.20 min).

the pseudomolecular ion $[M + NH_4]^+$, fragment ions [MH – RCOOH]$^+$, and acylium ion [RCO + 74]$^+$. Fragment ion [MH – RCOOH]$^+$ represents the loss of one fatty acid from the protonated molecular ion $[M + H]^+$. The acylium ion [RCO + 74]$^+$ is generated by the combined loss of one acyl group and one acyloxy moiety (Lauer et al., 1970; Itabashi et al., 1989). The pseudomolecular ion $[M + NH_4]^+$ was used to determine the molecular weight. Fragment ions [MH – RCOOH]$^+$ and acylium ion [RCO + 74]$^+$ were used to determine the pairing fatty acids in each triacylglycerol. Diacylglycerol with molecular weight M produces the pseudomolecular ions $[M + NH_4]^+$ and $[M + H]^+$, fragment ions [MH – 18]$^+$, and acylium ion [RCO + 74]$^+$. Fragment ion [MH – 18]$^+$ represents the protonated molecular ion loss of one water molecule and can be used to indicate the presence of diacylglycerol.

An example of a positive chemical ionization total ion chromatogram (PCI-TIC) of HPLC fraction Fr-E and one of its corresponding mass spectrum is shown in Figure 4. A total of 55 components were identified by the PCI mass

Table 1. Examples of SALATRIM 23CA Components with Pseudomolecular and Fragment Ions

acylglycerol[a]	fatty acids[a]	[M + 18]+	[MH − 18]+	[MH − RCOOH]+	[RCO + 74]+	acyl-glycerol[a]	fatty acids[a]	[M + 18]+	[MH − RCOOH]+	[RCO + 74]+
Ac–P–Ac	Ac, P	432		355	313	Ac–Ac–Lig	Ac, Lig	544	467	425
Ac–Ac–P	Ac, P	432		355	313	Ac–P–S	Ac, P, S	656	355, 383, 579	313, 341
Ac–P–Pr	Ac, Pr, P	446		355, 369	313	P–Ac–S	Ac, P, S	656	355, 383, 579	313, 341
Ac–Pr–P	Ac, Pr, P	446		355, 369	313	Pr/P/S	Pr, P, S	670	369, 397, 579	313, 341
Ac–S–OH	Ac, S	418	383		341	Ac–S–S	Ac, S	684	607	341
Ac–S–AC	Ac, S	460		383	341	S–Ac–S	Ac, S	684	607	341
Ac–Ac–S	Ac, S	460		383	341	Ac–O–S	Ac, O, S	682	381, 383, 605	339, 341
Ac–O–Ac	Ac, O	458		381	339	S–Ac–S	Ac, S	684	383, 607	341
Ac–Ac–O	Ac, O	458		381	339	O–Ac–S	Ac, O, S	682	381, 383, 605	339, 341
Ac–S–Pr	Ac, S, Pr	474		383, 397	341	Pr–S–S	Pr, S	698	397, 607	341
Ac–Pr–S	Ac, S, Pr	474		383, 397	341	S–Pr–S	Pr, S	698	397, 607	341
Pr–S–Pr	Pr, S	488		397	341	Ac/S/Ar	Ac, S, Ar	712	383, 411, 635	341, 369
Pr–Pr–S	Pr, S	488		397	341	Ac/S/Be	Ac, S, Be	740	383, 439, 663	341, 369
Ac–Ar–Ac	Ac, Ar	488		411	369	S–S–S	S	908	607	341
Ac–Ac–Ar	Ac, Ar	488		411	369	Ac/Lin/Ac	Ac, Lin	456		
Ac–Ar–Pr	Ac, Pr, Ar	502		411, 425	369	Ac–Pr–O	Ac, Pr, O	472	381, 397	339
Ac–Pr–Ar	Ac, Pr, Ar	502		411, 425	369	Ac–Pr–Be	Ac, Pr, Be	530	439, 453	397
Ac–Be–Ac	Ac, Be	516		439	397	Ac–Be–Pr	Ac, Pr, Be	530	439, 453	397
Ac–Ac–Be	Ac, Be	516		439	397	Ac/Pr/Lig	Ac, Pr, Lig	558		
Ac–Lig–Ac	Ac, Lig	544		467	425					

[a] Fatty acid abbreviations: Ac, acetic acid; Pr, propionic acid; S, stearic acid; Ar, arachidic acid; Be, behenic acid; Lig, lignerceric acid; O, oleic acid; Lin, linoleic acid. Components with / between fatty acids indicates that the fatty acid is not positional sensitive in the acylglycerol molecule. Components with – between fatty acids indicates that the fatty acid is positional sensitive in the acylglycerol molecule.

spectrometry. Some identified compounds with their characteristic ions and fatty acids are summarized in Table 1.

Positional Isomer Assignment in HTCGC Chromatogram. In this study, the HTCGC analysis using a methyl 65% phenyl silicone column separates diacetylstearoylglycerol into two peaks with peak area ratio of 27.5–72.5. These species were confirmed by ^{13}C NMR as being, respectively, 1,3-diacetyl-2-stearoylglycerol (Ac–S–Ac) and the combined asymmetric isomers (Ac–Ac–S + S–Ac–Ac). The NMR resonances gave a mole ratio of 2.8–7.5. Ac–S–Ac has the shorter retention time. By the same approach, the ratio of 1,3-distearoyl-2-acetylglycerol (S–Ac–S) to (Ac–S–S + S–S–Ac) in the distearoylacetylglycerol sample was determined to be 1:2 by both NMR analysis and HTCGC analysis; 1,2-distearoyl-3-acetylglycerol (S–S–Ac) has the shorter retention time on the methyl 65% phenyl silicone column. These observations are consistent with the findings of Myher et al. (1988). From this, the following observations were made for the assignments of triacylglycerol positional isomers in the gas chromatographic separations.

(*1*) When the triacylglycerol contains two short-chain fatty acids ($<C_4$) and one long-chain fatty acid, the positional isomer with a long-chain fatty acid at the secondary (*sn*-2) position elutes before the isomer with a long-chain fatty acid at the primary (*sn*-1 and *sn*-3) positions.

(*2*) When the triacylglycerol contains one short-chain fatty acid ($<C_4$) and two long-chain fatty acids, the positional isomer with a short-chain fatty acid at the primary (*sn*-1 and *sn*-3) positions elutes before the isomer with a short-chain fatty acid at the secondary (*sn*-2) position.

Quantification Consideration of SALATRIM 23CA. The analysis of model triacylglycerol standards by a Chrompack SIM-DIST CB column and a Quadrex methyl 65% phenyl silicone column was used to examine the recovery of triacylglycerols in short nonpolar and polar column. The recovery of triacylglycerol model compounds analyzed by the short nonpolar Chrompack SIM-DIST CB column does not decrease with an increase in carbon number (up to CN 57). The recovery of triacylglycerol is independent of the sample size within the range analyzed.

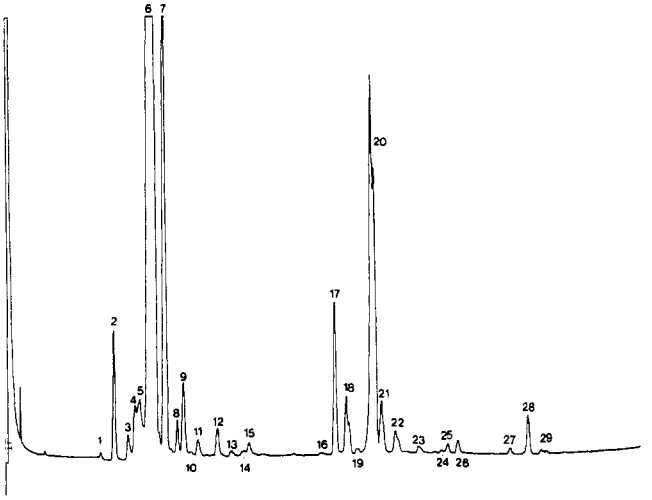

Figure 5. HTGCG (Chrompack SIM-DIST CB fused silica column) analysis of SALATRIM 23CA. The GC conditions are discussed under Experimental Procedures. Peak identifications are shown in Table 2.

On the other hand, the decreased recovery (response) with increasing carbon number (CN > 48) was observed in the analysis of model triacylglycerols using the methyl 65% phenyl silicone column. Triacylglycerols with CN < 42 did not show significant decreases with this analysis. The loss of higher molecular weight triacylglycerols depends on the amount of sample introduced.

Similar observations have been reported in other publications. Mares and Husek (1985) reported that the use of a cold on-column injection onto a 5 m long nonpolar capillary column yielded response factors for triacylglycerols with carbon numbers of 30–54 that are practically independent of the amount analyzed and the carrier gas flow rate. In another study, Gilkison (1989) reported the decrease of response with the increase of chain length and double bonds (CN > 42) when triacylglycerols were analyzed by the methyl 65% phenyl silicone column. No significant loss was observed for saturated triglyceride with CN ≤ 48. Gilkison also pointed out the loss is highly reproducible. The loss of higher molecular and highly unsaturated triacylglycerol depends on the amount of sample introduced. This dependence of triacylglycerol

Table 2. Weight Percent of SALATRIM 23CA Components [by Carbon Number (CN) and by Individual Acylglycerols]

by carbon number[a]			by acylglycerols[b]			
peak[c]	CN	wt %[d]	peak[e]	species identification	pk %[f]	wt %[g]
1	*h*	0.11	1	acetylpalmitoylglycerol	0.09	0.11
2	20	2.26	2	1,3-diacetyl-2-palmitoylglycerol	0.76	0.77
			3	1,2-diacetyl-3-palmitoylglycerol	1.47	1.49
3	21	0.58	4	2-palmitoylacetylpropionylglycerol	0.38	0.38
			5	1-palmitoylacetylpropionylglycerol	0.19	0.20
4 and 5	*h*	3.55	6	1,3-dipropionyl-2-palmitoylglycerol	0.06	0.09
			7	1,2-dipropionyl-3-palmitoylglycerol	trace	trace
			8	acetylstearoylglycerol	2.34	3.46
6	22	57.97	9	1,3-diacetyl-2-stearoylglycerol	25.19	19.24[i]
			10	1,2-diacetyl-3-stearoylglycerol	34.80	38.49[i]
			11	1,3-diacetyl-2-oleoylglycerol	0.19	0.18
			12	1,2-diacetyl-3-oleoylglycerol	0.06	0.06
				diacetyllinoleoylglycerol	trace	trace
7	23	11.50	13	2-stearoylacetylpropionylglycerol	8.72	7.67
			14	1-stearoylacetylpropionylglycerol	4.35	3.82
8 and 9	24	2.15	15	1,3-dipropionyl-2-stearoylglycerol	0.22	0.18
			16	1,2-dipropionyl-3-stearoylglycerol	0.49	0.41
			17	1,3-diacetyl-2-arachidoylglycerol	0.61	0.51
			18	1,2-diacetyl-3-arachidoylglycerol	1.20	1.00
			19	acetylarachidoylglycerol	0.05	0.04
10 and 11	25	0.42	20	2-arachidoylacetylpropionylglycerol	0.20	0.22
			21	1-arachidoylacetylpropionylglycerol	0.12	0.13
			22	unknown	0.06	0.07
12	26	0.67	23	1,2-dipropionyl-3-arachidoylglycerol	0.03	0.03
			24	unknown	0.09	0.07
			25	1,3-diacetyl-2-behenoylglycerol	0.24	0.18
			26	1,2-diacetyl-3-behenoylglycerol	0.51	0.40
13	27	0.16	27	2-behenoylacetylpropionylglycerol	0.09	0.09
			28	1-behenoylacetylpropionylglycerol	0.04	0.04
			29	unknown	0.03	0.03
14 and 15	28	0.43	30	1,3-diacetyl-3-lignoceroylglycerol	0.13	0.12
			31	1,2-diacetyl-3-lignoceroylglycerol	0.22	0.19
			32	2-lignoceroylacetylpropionylglycerol	0.03	0.02
			33	1-lignoceroylacetylpropionylglycerol	trace	trace
			34	unknown	0.08	0.07
			35	unknown	0.04	0.03
16	34	0.04	36	1,2-dipalmitoyl-3-acetylglycerol	0.05	0.04
				1,3-dipalmitoyl-2-acetylglycerol	trace	trace
17	IS	NA	37	internal standard	NA	NA
18	36	1.22	38	1-acetylpalmitoylstearoylglycerol	1.01	0.83
			39	2-acetylpalmitoylstearoylglycerol	0.48	0.39
19	37	0.09	40	1-propionylpalmitoylstearoylglycerol	0.10	0.05
			41	2-propionylpalmitoylstearoylglycerol	0.06	0.04
20	38	11.88	42	1,2-distearoyl-3-acetylglycerol	8.33	7.58
			43	1-acetyloleoylstearoylglycerol	0.43	0.39
			44	1,3-distearoyl-2-acetylglycerol	4.04	3.67
			45	2-acetyloleoylstearoylglycerol	0.26	0.23
21	39	0.93	46	1,2-distearoyl-3-propionylglycerol	0.78	0.57
			47	1,3-distearoyl-2-propionylglycerol	0.50	0.37
22	40	0.53	48	1-acetylarachidoylstearoylglycerol	0.33	0.35
			49	2-acetylarachidoylstearoylglycerol	0.16	0.17
23	41	0.17	50	1-propionylarachidoylstearoylglycerol	0.04	0.17
				2-propionylarachidoylstearoylglycerol	trace	trace
24 and 25	42	0.20	51	1-acetylstearoylbehenoylglycerol	0.09	0.14
			52	2-acetylstearoylbehenoylglycerol	0.04	0.06
				propionylstearoylbehenoylglycerol	trace	trace
26	44	0.22	53	1-acetylstearoyllignoceroylglycerol[j]	trace	0.22
				2-acetylstearoyllignoceroylglycerol	trace	trace
27	52	0.11	54	distearoylpalmitoylglycerol	0.05	0.11
28	54	0.71	55	tristearin	0.26	0.71
29	56	0.10		distearoylarachidoylglycerol[j]	trace	0.10

[a] Weight percent by carbon number (determined by SIM-DIST CB column). [b] Weight percent by acylglycerols (determined by methyl 65% phenyl silicone column). [c] Peak assignments in SIM-DIST CB column GC analysis; detailed in formation listed in Figure 5. [d] Weight percent (average of four measurements) by SIM-DIST CB column; calculations are described under Experimental Procedures. [e] Peak assignments in methyl 65% phenyl silicone GC analysis; detailed information shown in Figure 3. [f] Peak area percent (average of four measurements) determined by the methyl 65% phenyl silicone column; internal standard data were excluded from the calculation. [g] Weight percent of Salatrim 23CA. Calculations are shown under Experimental Procedures; the integrated peaks accounted for 96% by weight of the total injected sample. [h] Peak contains components other than triacylglycerols. [i] Ratio of 1,3-diacetyl-2-stearoylglycerol and 1,2-acetyl-3-stearoylglycerol was not integrated correctly with 1000 ppm sample. The ratio of the two positional isomers was determined to be 1 to 2 with 100 ppm sample. [j] Components were integrated in the SIM-DIST CB column analysis but were not integrated in the methyl 65% phenyl silicone column analysis.

recovery on the weight of the analyzed substance has been described by Mares and Husek (1985). The losses of higher unsaturated substances are greater in the polar column than on the shorter nonpolar column.

Precision of Normalized Peak Areas for SALATRIM 23CA Using the Quadrex Methyl 65% Phenyl Silicone Column and Chrompack SIM-DIST CB Column. The precision of the analysis by HTCGC was

determined from quadruplicate injections of SALATRIM 23CA under the HTCGC conditions set for both the Quadrex methyl 65% phenyl silicone column and the Chrompack SIM-DIST CB column. For SALATRIM 23CA analyzed by the Quadrex methyl 65% phenyl silicone column, components with normalized peak areas greater than 5.0% have less than 1% coefficient of variation (CV). Components with peak areas between 5.0% and 0.5% have CV ranges from 0.2% to 3%, and those peak areas of less than 0.5% have CVs that can be greater than 10%. This shows the peak area percent in SALATRIM 23CA HTCGC analysis is highly reproducible except for components of less than 0.5%.

For HTCGC analysis by the Chrompack SIM-DIST CB column, SALATRIM 23CA components with normalized peak areas greater than 10% have less than 1% CV. Normalized peak areas between 10% and 2% had CV ranges from 0.4% to 9%; peaks below 2% had CV values greater than 10%. The precision of triacylglycerols analyzed by a short nonpolar column in this study is well above the guideline set by the IUPAC standardized method for fat analysis (IUPAC, 1985).

Quantification Using the Chrompack SIM-DIST CB Column and the Quadrex Methyl 65% Phenyl Silicone Column. HTCGC analyses of SALATRIM 23CA using the Quadrex methyl 65% phenyl silicone column and the Chrompack SIM-DIST CB column are shown in Figures 3 and 5, respectively. A short nonpolar capillary column has the benefit of better triacylglycerol recovery and offers a wider linear range for triacylglycerol quantitation. On the other hand, a polar capillary column has the benefit of superior triacylglycerol separation. Utilizing both advantages, the weight percent by carbon number was determined by the Chrompack SIM-DIST CB column GC analysis and the ratio of acylglycerols having the same carbon number was determined using the Quadrex methyl 65% phenyl silicone column.

The weight percentages of SALATRIM 23CA components are summarized in Table 2. All components integrated by HTCGC were expressed by weight percents. Components below the integration limit and components identified in HPLC fractions but not detected in the unfractionated SALATRIM 23CA were classified as trace. In this study, a total of 55 acylglycerol molecular species were identified and 53 components were quantified. Among the quantified species, triacylglycerols account for about 96% by weight and diacylglycerols account for the remaining 4%. Triacylglycerols with two short-chain fatty acids and one long-chain fatty acid account for 80% of the SALATRIM 23CA acylglycerols. Triacylglycerols with one short-chain fatty acid and two long-chain fatty acids account for 16% of the SALATRIM 23CA acylglycerols.

Acylglycerols detectable by the analysis represent about 96% of the material injected in the HTCGC. Those components that were not identified in this study could come from (1) components that are not detectable by this approach, for example, minerals and trace organic compounds too volatile for HTCGC, and (2) components with concentrations below the detection limits, e.g., β-sitosterol, campesterol, brassicasterol, tocopherols, and the very minor acylglycerols.

Conclusion. The characterization of intact triacylglycerols in fats and oils can be useful in determining the origin and quality of the material and provides vital information for the development of new products in the food industry. The traditional fat analysis by fatty acid composition profiling can never yield sufficient information to reveal the original combination of components in the lipid mixture. Analysis of intact triacylglycerols by any chromatographic method always faces the difficult compromise of sensitivity, speed, resolution, and sample stability because of both the physical and chemical properties of these lipids. In this study, a combination of reversed-phase HPLC and aluminum-clad high-temperature capillary GC in sequence was used to separate the SALATRIM 23CA components. An on-line coupling of HTCGC with mass spectrometry revealed the structure of 55 individual components. To obtain reliable quantitative information, a combination of a short Chrompack SIM-DIST nonpolar column and a 25-m Quadrex methyl 65% phenyl silicone column was used for quantification. The qualitative and quantitative information obtained from this study is consistent with the theoretical values published by Klemann et al. (1994) and with NMR analysis of SALATRIM 23CA (Henderson et al., 1994).

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