Quantification of Generic Salatrim in Foods Containing Salatrim and Other Fats Having Medium- and Long-Chain Fatty Acids

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The methodology used in the quantification of various Salatrim mixtures in foods manufactured in combination with coconut oil, cocoa butter, and common vegetable oils is described. Salatrim is an interesterification product of triacetin, tripropionin, tributyrin, and hydrogenated vegetable oils. Approximately 95% by weight of Salatrim components are triacylglycerols with acyl carbon number (ACN) less than 42. In order to evaluate the method to measure Salatrim at various concentrations in foods containing other fats with similar ACN, 12 chocolate-coated peanut butter cup samples were made with several types of Salatrim, coconut oil at different concentrations, and other common vegetable oils and analyzed. The extraction of the crude fat from the samples by supercritical fluid carbon dioxide without the alteration of the triacylglycerol structures, separation of Salatrim from other lipids, and quantification by high-temperature capillary gas chromatography (HTCGC) with a short SIM-DIST fused-silica capillary column GC analysis is described. The triacylglycerols were characterized by HTCGC with positive chemical ionization mass spectrometry.

Keywords: *Salatrim; triglyceride; triacylglycerols; HTCGC; HTCGC-PCI-MS; SFE; low-calorie triglyceride; low-calorie triacylglycerols*

INTRODUCTION

Salatrim (*short and long acyl triglyceride molecule*) is a family of low-calorie triacylglycerols developed by Nabisco Inc. Salatrims are primarily triacylglycerols containing two short-chain fatty acids and one long-chain fatty acid and triacylglycerols containing two long-chain fatty acids and one short-chain fatty acid (Huang et al., 1994a; Softly et al., 1994). The detailed chemistry of Salatrim has been published by Klemann et al. (1994). Salatrim represents a new class of reduced calorie fat which delivers between 4.7 and 5.1 kcal/g instead of 9 kcal/g in common vegetable oils (Finley et al., 1994). It is important for food processors to characterize and quantify Salatrim from other fat sources in foods. To quantify Salatrim in foods, Huang et al. (1994b) used supercritical fluid carbon dioxide to quantitatively extract the crude fat containing Salatrim 23CA, followed by HPLC analysis with an evaporative light-scattering detector. (Various Salatrims are named as follows: the numbers refer to the short-chain fatty acid(s) material (i.e., 2, acetate; 3, propionate; 4, butyrate) followed by an indication of the source of the hydrogenated vegetable oil supplying stearic acid (i.e., CA, canola oil; SO, soybean oil).) The analysis requires that the same Salatrim be used as reference compound. Recently, Huang et al. (1995) published a method using the combination of supercritical fluid extraction (SFE) and high-temperature gas chromatography to quantify Salatrim in foods without using a reference Salatrim. These analytical methodologies depend on (1) the exhaustive extraction of the crude fat without altering intact triacylglycerol structures, (2) the effective resolution of all or part of the Salatrim triacylglycerols from the other fat sources, and (3) the correlation of individual triacylglycerols to their corresponding fat source in chromatographic profiles.

For the exhaustive crude fat extraction, Huang et al. (1994b, 1995) extracted various Salatrim mixtures from confectionery products and baked products without altering the Salatrim triacylglycerol structures by using SFE. The advantage of SFE over the traditional procedures for total fat extraction, such as acid/alkaline hydrolysis and Soxhlet extraction, has been repeatedly reported in the literature (Wright et al., 1988; Friedrich et al., 1982). The SFE fat extraction methods use milder extraction conditions and reduced extraction time. The resulting crude fat extract has less contamination with artifacts and is more suitable for the subsequent triacylglycerol analysis.

In order to separate all or part of the Salatrim triacylglycerols from other common fats and oils, Huang et al. (1994b) used reversed phase high-performance liquid chromatography (HPLC) with light-scattering detector to quantify Salatrim 23CA in the presence of other vegetable oils. Later, Huang et al. (1995) used a short nonpolar SIM-DIST column to separate and quantify various Salatrims in the presence of vegetable oils and butter fat. Of all the chromatographic techniques, gas liquid chromatography (GLC) is the most efficient for lipid separation and quantitation (Kuksis and Myher 1987). The use of polar columns, such as the 65% phenylmethylsilicone capillary column, and nonpolar columns, such as the OV-1 column, in triacylglycerol separation has been discussed in many publications (Monseigny et al., 1979; Mares and Husek, 1985; David et al., 1994). The short nonpolar columns separate triacylglycerols based on the total acyl carbon number (ACN). This type of column offers greater recovery and better quantification for triacylglycerols. High-temperature gas chromatography (HTGC) using short packed and capillary columns has been recommended to provide quantitative profiles of common fats and oils by the IUPAC (IUPAC, 1985). Salatrim triacylglycerols contain predominant saturated long- and short-chain fatty acids with ACN less than 42. The short nonpolar GC column is an excellent choice as the separation and quantification chromatographic tool for Salatrim. The other advantage of lipid analysis by GC

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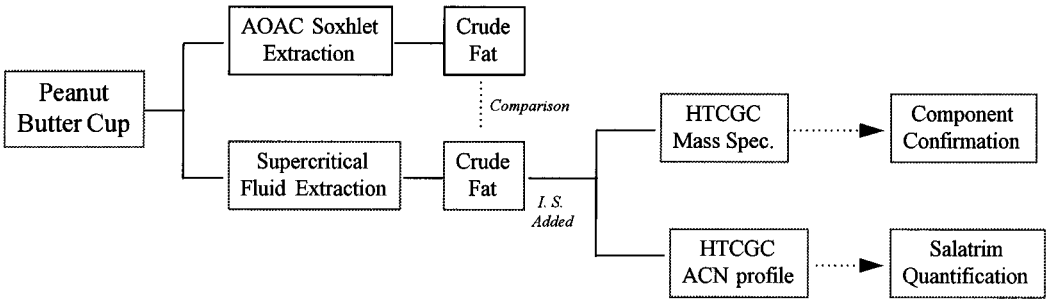

Figure 1. Quantification of Salatrim in foods made with Salatrim, coconut oil, and other common vegetable oils.

is the easy coupling of GC with mass spectrometry. Mass spectrometry with ammonia chemical ionization has been used in the identification of triacylglycerols in several laboratories (Murata, 1977; Murata and Takahashi, 1977; Oshima et al., 1989; Huang et al., 1994a). More recently, Huang et al. (1995) used a short SIM-DIST capillary column GC combined with ammonia chemical ionization mass spectrometry to confirm various Salatrim components.

Salatrim has a wide range of applications in foods including baked, confectionery, and dairy products. Fats and oils containing similar ACN, such as butter fat and coconut oil, can be used in combination with Salatrim in some food applications. In a previous study (Huang et al., 1995), we described an approach for the quantification of Salatrim in the presence of butter fat. In this study, we report on an analytical method for the quantification of Salatrim in foods made with coconut oil, Salatrim, and other common vegetable oils without using a reference Salatrim mixture. This report includes the following: (1) the use of SFE in the quantitative extraction of crude fat containing various Salatrims, coconut oil, and other fats and oils from foods; (2) the use of a nonpolar short column to separate the crude fat triacylglycerols according to their ACN; (3) the use of high-temperature capillary gas chromatography (HTCGC) with positive chemical ionization mass spectrometry to confirm the Salatrim components; and (4) the quantification of Salatrim in foods by measuring the triacylglycerols having one short- and two long-chain fatty acids and triacylglycerols having two short-chain fatty acids with one long-chain fatty acid.

EXPERIMENTAL PROCEDURE

An outline of the experimental procedure used in this study is shown in Figure 1.

Materials. Twelve different chocolate-coated peanut butter cup samples were analyzed. Each sample was manufactured with various combinations of the following oils: Salatrim 4SO, Salatrim 23SO, Pureco 100 hydrogenated coconut oil, peanut oil, cocoa butter, rapeseed oil, cottonseed oil, and soybean oil. Salatrim 4SO and Salatrim 23SO were obtained from the Nabisco Food Group Special Projects Group, East Hanover, NJ. Pureco 100 hydrogenated coconut oil was obtained from Abitec Corp., Columbus, OH. Peanut oil originated from the peanut flour supplied by the Golden Peanut Co., Atlanta, GA. Partially hydrogenated cottonseed oil, rapeseed oil, and soybean oil were ingredients of Lipodan SET-K, which was obtained from Danisco Inc., Kansas City, MO. The 12 peanut cup samples were manufactured at the Nabisco Inc. Margarine Pilot Plant at Indianapolis, IN.

Each of the 12 samples was made with 40% by weight peanut butter center and 60% by weight chocolate coating. The chocolate coating was made with 47.6% granulated sugar, 34.0% added fat (hydrogenated coconut oil and Salatrim 23SO), 18.0% cocoa powder, 0.4% lecithin, and 0.03% vanillin. The peanut butter center contained 53.0% peanut flour, 27.0% added fat (hydrogenated coconut oil and Salatrim 4SO), 10.0% sugar, 7.5% nonfat dry milk powder, 1.2% salt, and 1.3% Lipodan SET-K. The compositions and weight percentages of fat added in the chocolate coating for each of the 12 samples were as follows: sample 1, 34% hydrogenated coconut oil; sample 2, 34% hydrogenated coconut oil; sample 3, 20.3% hydrogenated coconut oil and 13.7% Salatrim 23SO; sample 4, 30.6% hydrogenated coconut oil and 3.4% Salatrim 23SO; sample 5, 27.2% hydrogenated coconut oil and 6.8% Salatrim 23SO; sample 6, 23.8% hydrogenated coconut oil and 10.2% Salatrim 23SO; sample 7, 20.4% hydrogenated coconut oil and 13.6% Salatrim 23SO; sample 8, 16.9% hydrogenated coconut oil and 17.1% Salatrim 23SO; sample 9, 23.8% hydrogenated coconut oil and 10.2% Salatrim 23SO; sample 10, 3.4% hydrogenated coconut oil and 30.6% Salatrim 23SO; sample 11, 3.4% hydrogenated coconut oil and 30.6% Salatrim 23SO; sample 12, 34.0% Salatrim 23SO. The make-up of fat added in the peanut butter center for each of the 12 samples were as follows: sample 1, 27.0% hydrogenated coconut oil; sample 2, 34% hydrogenated coconut oil and 10.8% Salatrim 4SO; sample 3, 27.0% hydrogenated coconut oil; sample 4, 24.2% hydrogenated coconut oil and 2.8% Salatrim 4SO; sample 5, 21.6% hydrogenated coconut oil and 5.4% Salatrim 4SO; sample 6, 21.6% hydrogenated coconut oil and 5.4% Salatrim 4SO; sample 7, 16.2% hydrogenated coconut oil and 10.8% Salatrim 4SO; sample 8, 13.3% hydrogenated coconut oil and 13.3% Salatrim 4SO; sample 9, 8.2% hydrogenated coconut oil and 18.8% Salatrim 4SO; sample 10, 5.5% hydrogenated coconut oil and 21.5% Salatrim 4SO; sample 11, 24.4% hydrogenated coconut oil and 2.8% Salatrim 4SO; sample 12, 34% Salatrim 4SO.

Determination of Fatty Acids. Coconut oil was analyzed for its fatty acids by gas chromatography of the fatty acid methyl esters (FAME). The transesterification of coconut oil triacylglycerols into their respective methyl esters was performed using a reagent of boron trifluoride in methanol (BF_3/methanol, 14/100, w/v, purchased from Alltech Associates, Deerfield, IL) with a 0.5 N methanolic sodium hydroxide solution. The transesterification was performed according to the procedure described by Athnasios et al. (1986). The gas chromatographic analysis of FAME was performed using a Hewlett-Packard Model 5890 series II gas chromatography equipped with a flame ionization detector (FID). A Supelco SP2560 capillary column (100 m × 0.25 mm i.d. with 0.2 μm film thickness obtained from Supelco Inc., Supleco Park, Bellfonte, PA) was used for the separation. The injector and detector temperatures were both set at 275 °C. The initial oven temperature was set at 178 °C for 23 min, followed by ramping the temperature from 178 to 200 °C at 4 °C/min, and then held at 200 °C for 22 min. The helium flow rate was set at 20 cm/s. A mixture of standard methyl esters, containing equal weights of methyl caproate, methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, methyl arachidate, and methyl behenate was used for comparison. All methyl ester standards were obtained from Alltech Associates.

Total Crude Fat As Determined by the AOAC Standard Procedure. The fat content of the 12 samples was

determined according to the AOAC Soxhlet extraction method 27.006 (AOAC, 1984). Each sample was analyzed in triplicate. The fat content was expressed in weight percent of the corresponding food item.

Crude Fat Extraction by the SFE Procedure. Each sample was ground to a uniform paste, and 0.25−0.3 g of sample was used for each SFE extraction. At the end of each extraction, the sample in the thimble was weighed and the weight loss was determined. The fat collected in the stainless steel trap was washed into a preweighed empty vial with hexane. The hexane was removed using a stream of nitrogen, and the fat was weighed before use in the subsequent analysis. The crude fat content (C_{FAT}) was expressed as the weight percent of fat in the corresponding sample. The supercritical carbon dioxide extraction was performed with a Hewlett-Packard 7680T extraction module (Hewlett-Packard, Palo Alto, CA) and the conditions were as follows.

Extraction Conditions. Approximately 0.25−0.3 g of sample was placed into a 7.0 mL stainless steel extraction cell and held in place by glass wool plugs at both ends. A glass rod (6 cm long × 1 cm o.d.) was placed inside the extraction cell to reduce the void volume. The extraction cell was placed in the extraction chamber and the temperature was maintained at 80 °C. Anaerobic grade carbon dioxide (JWS Technology Inc., Piscataway, NJ) was used as the extraction fluid. CO_2 was maintained at a density of 0.8 g/mL, pressure at 365 bar, and a flow rate of 4 mL/min. After the density and temperature were reached, the sample was soaked in the SF CO_2 for an equilibration time of 2 min without CO_2 flowing through the chamber. After the equilibration time ended, the CO_2 fluid flowed through the sample thimble for 45 min to carry the crude fat into the analyte trap.

Trap Conditions. A stainless steel trap was maintained at 5 °C. The nozzle temperature was kept at 45 °C.

Fraction Output. Hexane (3 × 1 mL) at a flow rate of 1.0 mL/min was used as the rinse solvent. The nozzle and trap temperatures at the rinsing stage were both kept at 30 °C.

Analysis of the Standards and Salatrim by Chrompack SIM-DIST CB Capillary Column HTCGC. *Solvent.* Both undecane and toluene were spectrometric grades and were obtained from the Aldrich Chemical Co., St. Louis, MO.

Triglyceride Standard. Thirteen triacylglycerol standards (tricaproin, triheptanoin, tricaprylin, trinonanoin, tricaprin, triundecanoin, trilaurin, tritridecanoin, trimyristin, tripentadecanoin, tripalmitin, triheptadecanoin, tristearin) were obtained from Nu Chek Prep, Inc., Elysian, MN. The purity was greater than 99%.

Stock Solution. The stock solution was prepared by dissolving 100 mg of triundecanoin as internal standard in 1 L of undecane/toluene (95/5, v/v).

Triglyceride Standard and Reference Coconut Oil Solution. (1)The triglyceride standard solution contained approximately the same concentration (100 μg/mL) of the following triacylglycerol standards in undecane/toluene (95/5, v/v): tricaproin (ACN 18), triheptanoin (ACN 21), tricaprylin (ACN 24), trinonanoin (ACN 27), tricaprin (ACN 30), triundecanoin (ACN 33), trilaurin (ACN 36), tritridecanoin (ACN 39), trimyristin (ACN 42), tripentadecanoin (ACN 45), tripalmitin (ACN 48), triheptadecanoin (ACN 51), and tristearin (ACN 54). (2) The reference coconut oil solution contained ∼4000 mg/L coconut oil in stock solution.

Extracted Crude Fat Solutions for Salatrim Quantification. Each test solution contained ∼4000 mg/L extracted crude fat in the stock solution (with internal standard). Crude fat extracts were obtained from the SFE of the 12 food samples. Each test solution contained 100 ppm (μg/mL) of triundecanoin as internal standard.

Analysis of Triacylglycerols, Coconut Oil, and Extracted Crude Fats by HTCGC. Analyses of the triacylglycerol standard, coconut oil, and crude fat solutions followed the procedures described by Huang et al. (1995): The crude fat solution was filtered through an Acrodisc CR PTFE syringe filter (Scientific Resources Inc., Eatontown, NJ) before the HTCGC analysis. The analyses were performed using a Hewlett-Packard (HP) 5890 Series II GC system. The system was equipped with a flame ionization detector, electronic pressure control (EPC) on-column injector, HP 7673 autosampler (with a nanoliter adapter and a 5 μL syringe), and HP DOS ChemStation data system (Hewlett-Packard Co.). The separation was accomplished with a Chrompack SIM-DIST CB fused silica column (4.8 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 Corp., New Haven, CT). The oven temperature was ramped from 100 to 150 °C at 15 °C/min and then from 150 to 345 °C at 10 °C/min and held at 345°C for 5 min. Hydrogen was used as the carrier gas with a constant flow rate operating at 5.5 psi (measured at 140 °C). The FID was operated at 375 °C. On-column injection was used with the injector operated in the temperature track mode. An injection volume of 0.1 μL was used for the analysis.

Identification of the Extracted Crude Fat 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 5989A mass spectrometer was used for the analysis. The HP 5890 GC was equipped with an electronic pressure controlled on-column injector and a HP7673 autosampler.

Gas Chromatography. A Chrompack SIM-DIST CB fused-silica column (10 m × 0.32 mm i.d., 0.1 μm film thickness, Chrompack Inc.) was used for separation. A deactivated fused-silica precolumn of 0.8 m × 0.53 mm i.d. was coupled to the analytical column via a butt connector (Quadrex Corp.). The oven temperature was ramped from 50 to 150 °C at 18 °C/min and then from 150 to 350 °C at 12 °C/min and held at 350 °C for 10 min. On-column injection was performed in the temperature track mode. An injection volume of 1.0 μL was used for the analysis. Helium was used as the carrier gas with a constant flow rate operating at the column head pressure of 5 psi (measured at 100 °C).

GC−MS Interface. The interface between the GC and mass spectrometer was maintained at 350 °C.

Mass Spectrometry. The source temperature was set at 275 °C. Ammonia was used as the reagent gas at a source pressure of 1.8 Torr. The mass to charge ratios (m/z) of the various peaks were determined by the mass marker which had been calibrated with poly(propylene glycol) (PPG; Hewlett Packard Co.). A scan range of m/z 150−1000 was used. A HP UNIX ChemStation software package was used for data acquisition and data processing.

Determination of the Relative Response Factors of Standard Triacylglycerols and the Establishment of a Calibration Curve (Relative Response Factor vs Retention Time). The relative response factors of the standard triacylglycerols were determined by the analysis of standard triacylgycerol solutions. The procedure is as follows: Thirteen triglyceride standards representing different ACN were used. They were tricaproin, triheptanoin, tricaprylin, trinonanoin, tricaprin, triundecanoin, trilaurin, tritridecanoin, trimyristin, tripentadecanoin, tripalmitin, triheptadecanoin, and tristearin. Triundecanoin was used as the internal standard. The relative response factor to triundecanoin (RF_i) for each of the standard triacylglycerols of ACN i at retention time t was determined by the following equation:

$$RF_i = (C_{IS}/C_i)(A_i/A_{IS}) \quad (1)$$

where A_{IS} is the peak area counts of triundecanoin, A_i is the peak area counts of the standard triacylglycerol (i), C_i is the concentration (μg/mL) of the standard triacylglycerol (i), and C_{IS} is the concentration (μg/mL) of triundecanoin.

The retention time (t) of each standard triacylglycerol (i) with its corresponding relative response factor was used to establish the calibration curve.

Determination of the Relative Response Factors of Triacylglycerol Components and Their Weight Percents in the Crude Fat Extract and in the Coconut Oil Solution. The relative response factors and the weight percent of each triacylglycerol in the crude fat and in coconut oil were obtained from the analyses of the crude fat and coconut oil

solutions. The concentration (μg/mL) of each triacylglycerol of ACN i (C_i) in the solution was calculated by the following equation:

$$C_i = (C_{IS}/RF_i)(A_i/A_{IS}) \quad (2)$$

where A_{IS} is the peak area counts of triundecanoin, A_i is the peak area counts of the triacylglycerol (i), C_i is the concentration (μg/mL) of the triacylglycerol component (i) at retention time (t), C_{IS} is the concentration (μg/mL) of internal standard triundecanoin, and RF_i is the relative response factor of triacylglycerol component with ACN i in the solution. The relative response factor of component i (at retention time t) was determined from the calibration curve established in the previous section.

The weight percent of each triacylglycerol with ACN i (WT_i) in the crude fat extract or in the coconut oil was calculated as follows:

$$T_i = (C_i/C_T) \times 100 \quad (3)$$

where C_T is the known concentration of crude fat or coconut oil in the test solution.

Quantification of Salatrim in the 12 Samples. Determination of the Ratio (R_{C3234}) between the Combined Weight of Coconut Oil Triacylglycerols with ACN 20−42 and the Combined Weight of Coconut Oil Triacylglycerols with ACN 32 and ACN 34 from the Analysis of the Reference Coconut Oil Solution. From the HTCGC analysis of the coconut oil solution, the ratio (R_{C3234}) of the combined weight of coconut oil triacylglycerols to ACN 20−42 ($WTC_{ACN20-42}$) and the combined weight of coconut oil triacylglycerols at ACN 32 and ACN 34 ($WTC_{ACN32,34}$) was calculated as follows:

$$R_{C3234} = WTC_{ACN20-42}/(WTC_{ACN32} + WTC_{ACN34}) = \sum_{i=20}^{42} WT_i/WT32,34 \quad (4)$$

Quantification of Salatrim in Foods. The levels of Salatrim in the 12 samples were calculated from the HTCGC analysis of the corresponding crude fat sample. Salatrim weight was determined by subtracting the total weight of coconut oil components with ACN 20−42 from the total weight of components with ACN 20 to 42. The equation is as follows:

$$WT_{SAL} = (WTA_{CN20-42} - WTC_{ACN20-42}) \times 100/95$$

$$= (\sum_{i=20}^{42} WT_i - WT_{32,34}R_{C3234}) \times 1.053 \quad (5)$$

$$C_{SAL} = WT_{SAL}C_{FAT} \times 100 \quad (6)$$

where $WT_{ACN20-42}$ is the total weight of triacylglycerol with ACN from 20 to 42 in the crude fat extract; $WTC_{ACN20-42}$ is the total weight of coconut oil triacylglycerols having ACN from 20 to 42 in the crude fat extract. R_{C3234} was obtained from coconut oil analysis and calculated from eq 4; C_{SAL} is the weight percent of Salatrim in foods; WT_{SAL} is the weight percent of Salatrim in the extracted crude fat; C_{FAT} is the weight percent of crude fat in foods as determined by the supercritical fluid crude fat extraction.

Statistical Analysis of Crude Fat and Salatrim in Foods. The standard deviations, percent coefficients of variation (% CV), and correlation coefficients (R^2) between the analytical results and the theoretical values were calculated according to the AOAC (Wernimont, 1985).

RESULTS AND DISCUSSION

Comparison of Total Crude Fat Content As Determined by the AOAC Soxhlet and Supercritical Fluid Extraction Methods. Soxhlet extraction is recommended for both chocolate and peanuts as the total fat extraction method by the AOAC. The 12 samples, combinations of chocolate and peanuts, were extracted by the Soxhlet method, and the results were used as the reference for the level of total fat. The percent fat in each sample as determined by the AOAC Soxhlet extraction and SFE methods are shown in Table 1. The precision of the SFE method is good as indicated by the coefficients of variation, which ranged from less than 1 to 3.9%. The % CV for the AOAC method ranged from 0.03 to 1.8%. These precision data were similar to the results obtained in our previous studies (Huang et al., 1995)

The SFE results ranged from 97 to 102% when compared to the AOAC Soxhlet results, confirming that all of the fat had been extracted by the SFE method. This corroborated that the SFE method is comparable to the Soxhlet extraction for this type of product. Since, in this study, the final analysis is based on the quantification of intact triacylglycerols, the fat extraction must not alter the triacylglycerol structures. Traditional exhaustive crude fat extraction often involves acid/alkaline hydrolysis, multistep extraction procedures, and Soxhlet extraction with long extraction time. The crude fat extraction procedure involving acid hydrolysis is prone to the formation of artifacts (Carpenter et al., 1993). The Soxhlet extraction requires a long extraction time. SFE utilizes much milder extraction conditions and a short extraction time. The crude fats isolated by the SFE were used for the consequent analyses.

Analysis of the Crude Fat Extracts According to Their Acyl Carbon Number Using a Short Nonpolar Column. Triacylglycerol separation by ACN by the short nonpolar column, such as the OV-1 column and SIM-DIST CB column, HTCGC has been known for some time and has been recommended to provide quantitative profiles of fats and oils by IUPAC (IUPAC, 1985). This type of analysis is used routinely for quality control by chocolate manufacturers and related food processors (David, et al., 1994). In a recent publication, David et al. (1994) demonstrated the high reproducibility of milk fat/cocoa butter ACN profiling using an automated cool on-column injection and 5 m OV-1 capillary column. In the previous study, Huang et al. (1995) evaluated the recovery of triacylglycerols with ACNs from 18 to 54 on a 5 m SIM-DIST column. The results indicate that the response factors for triglycerides with ACNs of 18−54 are independent of quantities of injection for triacylglycerols between the range of 8 to over 2000 ng (per peak). The recovery of triacylglycerol model compounds (ACN 18−54) analyzed by the short nonpolar Chrompack SIM-DIST CB column does not decrease with the increase in acyl carbon number up to ACN 54. This type of analysis is particularly good for profiling Salatrim with ACN mostly within the range of 20−42 and with primarily saturated fatty acids. In this study, a 4.8 m SIM-DIST capillary column was used to separate sample triacylglycerols according to ACN. The ACNs and their fatty acid moieties were confirmed by HTCGC-PCI-MS.

Separation and Quantification of Salatrim Triacylglycerols from Other Fats by Short Nonpolar Column Chromatography. Salatrim contains mostly triacylglycerols having two long-chain fatty acids and one short-chain fatty acid and triacylglycerols having two short-chain fatty acids and one long-chain fatty acid. The short-chain fatty acids are acetic acid (C2:0), propionic acid (C3:0), and butyric acid (C4:0). The long-

Table 1. Percent Fat As Determined by the AOAC Soxhlet and by the SFE Procedures

	sample											
	1	2	3	4	5	6	7	8	9	10	11	12
(A) AOAC Soxhlet Extraction[a]												
run 1	36.31	37.51	36.84	36.31	36.38	36.54	36.58	36.44	36.51	37.26	36.4	36.72
run 2	36.24	36.24	36.63	36.29	36.31	36.64	36.79	36.61	36.36	36.85	36.47	36.56
run 3	36.84	36.51	36.73	36.29	36.12	36.42	36.8	36.64	36.37	36.49	36.74	36.59
average	36.46	36.75	36.73	36.30	36.27	36.53	36.72	36.56	36.41	36.87	36.54	36.62
SD	0.33	0.67	0.11	0.01	0.13	0.11	0.12	0.11	0.08	0.39	0.18	0.09
% RSD	0.90	1.82	0.29	0.03	0.37	0.30	0.34	0.29	0.23	1.05	0.49	0.23
(B) SFE[b]												
run 1	35.67	36.52	35.85	37.75	37.92	37.01	36.1	35.86	36.64	36.99	36.52	36.97
run 2	36.49	36.1	35.5	36.12	35.27	35.76	36.17	36.93	37.55	36.03	36.18	38.71
run 3	36.45	36.85	35.35	35.86	35.71	35.31	36.86	36.45	37.33	35.9	37.31	36.31
average	36.20	36.49	35.57	36.58	36.30	36.03	36.38	36.41	37.17	36.31	36.67	37.33
SD	0.46	0.38	0.26	1.02	1.42	0.88	0.42	0.54	0.47	0.60	0.58	1.24
% RSD	1.28	1.03	0.72	2.80	3.91	2.44	1.15	1.47	1.28	1.64	1.58	3.32
% recovery[c]	99	99	97	101	100	99	99	100	102	98	100	102

[a] AOAC Soxhlet extraction data were expressed in weight percent of the corresponding sample. [b] SFE data were expressed in weight percent of the corresponding sample. [c] Percent recovery of fat by SFE was based on the assumption that the fat recovery by Soxhlet extraction was 100%.

chain fatty acids are predominantly stearic acid (C18:0) and palmitic acid (C16:0) with trace amounts of arachidic acid (C20:0) and oleic acid (C18:1). In Salatrim, practically all of the triacylglycerols are within the ACN 20−40 range. These groups account for ~95% by weight of Salatrim. Triacylglycerols found in common vegetable oils (such as soybean oil, sunflower oil, corn oil, and canola oil) have ACNs greater than 42, the majority being between ACN 48 and 56. Other commonly used oils, e.g., palm oil and cocoa butter, also have triacylglycerols with ACNs from 48 to 56. Huang et al. (1995) demonstrated that these common oils can be distinguished from Salatrim by differences in triacylglycerol acyl carbon numbers by HTCGC analysis with a short SIM-DIST column.

Some commercial fats and oils, such as coconut oil, contain significant amounts of medium-chain fatty acids, and their major components are triacylglycerols with ACNs less than 42. The triacylglycerols from these fats will coelute with Salatrim triacylglycerols in the HTCGC nonpolar column analysis. For example, Preco 100 hydrogenated coconut oil (used in this study) was determined to have the following fatty acid composition: 0.35% caproic acid (C6:0), 5.66% caprylic acid (C8:0), 5.27% capric acid (C10:0), 46.55% lauric acid (C12:0), 19.44% myristic acid (C14:0), 10.03% palmitic acid (C16:0), 12.45% stearic acid (C18:0), and 0.1% arachidic acid (C20:0). Coconut oil triacylglycerols have ACNs from 26 to 54, the majority being between ACN 32 and 44. Comparative HTCGC ACN profiles of Salatrim 23SO, Salatrim 4SO, and coconut oil are illustrated in Figure 2. Both Salatrim and coconut oil could have triacylglycerols with ACN 26, 28, 36, 38, and 40 depending upon the type of Salatrim used. On the other hand, coconut oil triacylglycerols also have some unique ACNs that are essentially absent from Salatrim and common vegetable oils. Some of these coconut oil triacylglycerols are found at ACN 32 and 34 as shown in Figure 2. Quantification of triacylglycerols with ACN 32 and 34 can be used to estimate the amount of coconut oil triacylglycerols with ACN between 20 and 42. The ratio (R_{C3234}) of the total weight of triacylglycerols with ACN 20−42 and combined weight of triacylglycerols of ACN 32 and 34 was calculated from the analysis of the reference coconut oil. The ratio (R_{C3234}) was calculated to be 3.2 for the reference coconut oil used in this study.

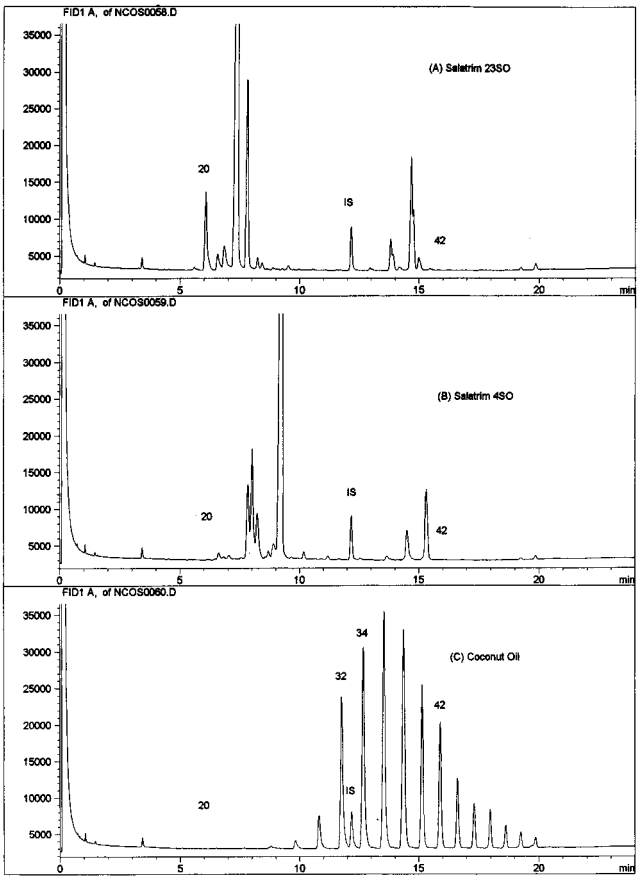

Figure 2. Comparative HTCGC profiles of (A) Salatrim 23SO, (B) Salatrim 4SO, and (C) Pureco 100 hydrogenated coconut oil. Numbers indicated on the figures are ACNs.

The percent Salatrim, percent coconut oil, and the weight ratios between Salatrim and coconut oil in each sample are summarized in Table 2. The HTCGC ACN profiles of crude fats isolated from samples 1 through 12 are shown in Figures 3−6. As seen in Figure 3A, sample 1 contained 31.2% coconut oil, peanut oil, cocoa butter, rapeseed oil, cotton seed oil, and soybean oil, but no Salatrim. These vegetable oils have triacylglycerols with ACN 48−58. From samples 4−11, the ratio of Salatrim to coconut oil increased, approximately, from 10/90 to 90/10. By comparing the HTCGC profiles of

Table 2. Weight Percentages of Individual Fats in 12 Peanut Butter Cup Samples[a]

sample	Salatrim 23SO wt %	Salatrim 4SO wt %	total Salatrim[b] wt %	total coconut oil wt %	Salatrim/ CNO[c]
1	0.00	0.00	0.00	31.20	0/100
2	0.00	4.32	4.32	26.88	13.8/86.2
3	8.23	0.00	8.23	22.98	26.4/73.6
4	2.04	1.10	3.14	28.06	10.1/89.9
5	4.10	2.18	6.28	24.92	20.1/79.9
6	6.12	3.26	9.38	21.82	30.1/69.9
7	8.17	4.31	12.48	18.72	40/60
8	10.22	5.42	15.64	15.55	50.1/49.9
9	14.24	7.57	21.80	9.38	69.9/30.1
10	16.27	8.62	24.89	6.31	78.8/20.2
11	18.36	9.68	28.05	3.15	89.9/10.1
12	20.39	10.81	31.20	0.00	100/0

[a] Besides Salatrim and coconut oil, other fats included peanut oil, cocoa butter, rapeseed oil, cotton seed oil, and soybean oil. All weight percents were calculated from the formulations (theoretical values). [b] The combined weight of Salatrim 4SO and Salatrim 23SO. [c] The weight ratio between Salatrim and coconut oil.

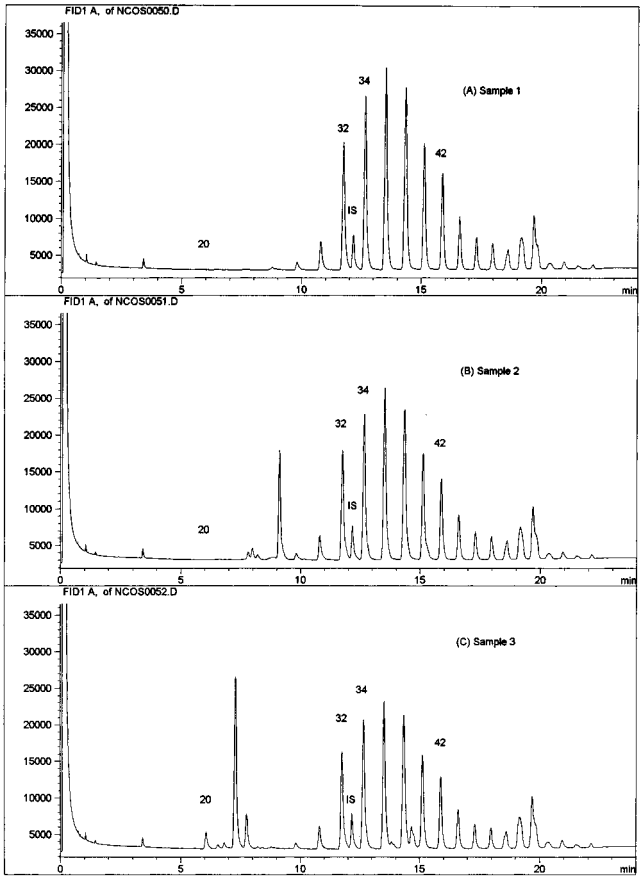

Figure 3. Comparative HTCGC profiles of crude fats isolated from peanut butter cup samples (A) 1, (B) 2, and (C) 3. Sample 1 contained 31.2% coconut oil and no Salatrim. Sample 2 contained 4.32% Salatrim and 26.88% coconut oil. Sample 3 contained 8.23% Salatrim and 22.98% coconut oil. All samples contained peanut oil, cocoa butter, cottonseed oil, rapeseed oil, and soybean oil. Numbers indicated on the figures are ACNs.

samples 4−11 (Figure 4−6, the increase in Salatrim components and the decrease in coconut oil components becomes apparent. Sample 12 contains 31.2% Salatrim and no coconut oil, and its corresponding chromatogram (Figure 6C) shows no coconut oil peaks.

Since triacylglycerols of ACN 32 and 34 in samples 2−11 were derived solely from coconut oil, the coconut oil triacylglycerols with ACN 20−42 in the crude fat can be calculated from the R_{C3234} ratio and the combined

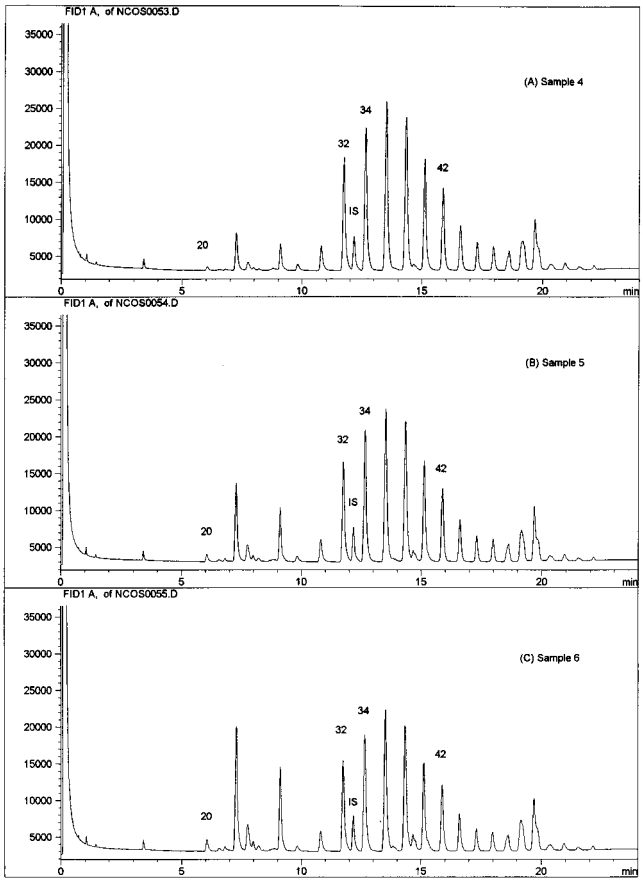

Figure 4. Comparative HTCGC profiles of crude fats isolated from peanut butter cup samples (A) 4, (B) 5, and (C) 6. Sample 4 contained 3.14% Salatrim and 28% coconut oil. Sample 5 contained 6.28% Salatrim and 24.9% coconut oil. Sample 6 contained 9.38% Salatrim and 21.8% coconut oil. All samples contained peanut oil, cocoa butter, cottonseed oil, rapeseed oil, and soybean oil. Numbers indicated on the figures are ACNs.

weight of triacylglycerols of ACN 32 and 34 in the crude fat extract. The total weight of triacylglycerols with ACN 20−42 in crude fat minus the amount of coconut oil with ACN 20−42 represents 95% by weight of Salatrim. In Salatrim, triacylglycerols with ACN 20−42 account for 95% by weight of the mixture. A correction factor of 1.053 (100/95) was used to reflect the true weight of Salatrim in this study.

Confirmation of ACN and Triacylglycerol Fatty Acid Moieties in HTCGC Peaks by HTCGC-PCI-MS. In positive [NH_3] CIMS spectrometry, a triacylglycerol with molecular weight M produces the pseudomolecular ion $[M + NH_4]^+$, fragment ions $[MH - RCOOH]^+$, and acylium ions $[RCO + 74]^+$. Fragment ion $[MH - RCOOH]^+$ represents the loss of one fatty acid from 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.

In this study, a short nonpolar capillary column was used to separate the crude fat triacylglycerols by acyl carbon number. Since each peak may contain more than one triacylglycerol, HTCGC-MS was used to confirm the ACN and its fatty acid. An example is illustrated in Figure 7. This figure shows the positive chemical ionization total ion chromatogram of the crude

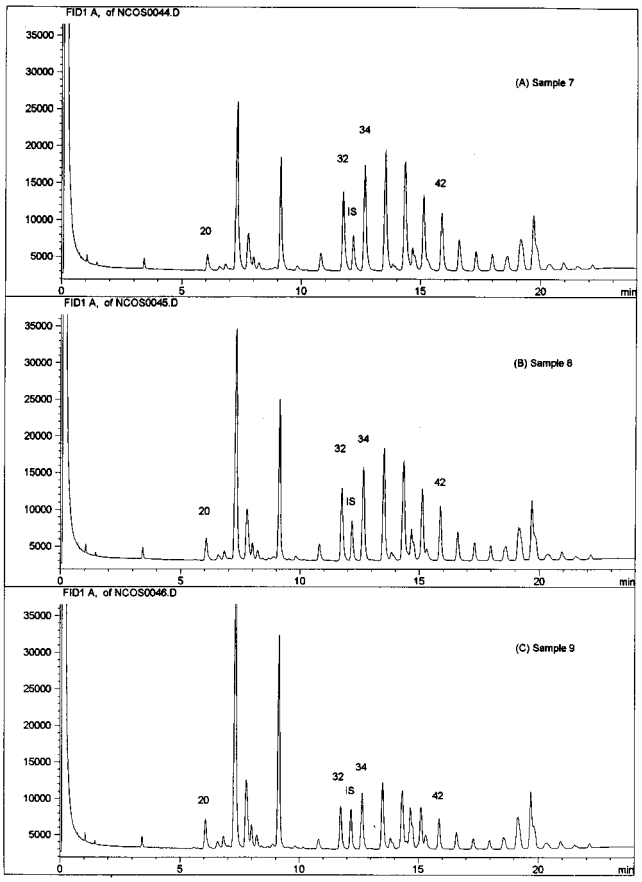

Figure 5. Comparative HTCGC profiles of crude fats isolated from peanut butter cup samples (A) 7, (B) 8, and (C) 9. Sample 7 contained 12.48% Salatrim and 18.72% coconut oil. Sample 8 contained 15.64% Salatrim and 15.5% coconut oil. Sample 9 contained 21.8% Salatrim and 9.38% coconut oil. All samples contained peanut oil, cocoa butter, cottonseed oil, rapeseed oil, and soybean oil. Numbers indicated on the figures are ACNs.

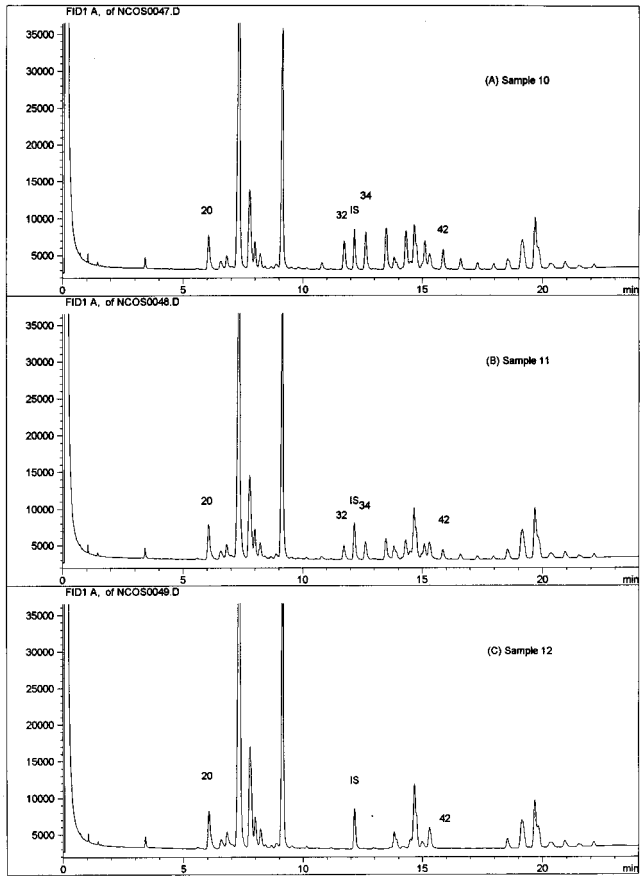

Figure 6. Comparative HTCGC profiles of crude fats isolated from peanut butter cup samples (A) 10, (B) 11, and (C) 12. Sample 10 contained 24.89% Salatrim and 6.31% coconut oil. Sample 11 contained 28.05% Salatrim and 3.15% coconut oil. Sample 12 contained 31.2% Salatrim and no coconut oil. All samples contained peanut oil, cocoa butter, cottonseed oil, rapeseed oil, and soybean oil. Numbers indicated on the figures are ACNs.

fat isolated from sample 6 and the PCI spectrum of the peak at a retention time of 17.53 min. The component had a pseudomolecular ion $[M + NH_4]^+$ of 600. The molecular weight was determined to be 582, which confirmed that the component has an ACN of 32. The peak has fragment ions $[MH - RCOOH]^+$ at m/z 439, 411, 383, 355, and 299. Respectively, these fragment ions indicate the losses of caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), and stearic acid (C18:0) from their corresponding protonated molecular ions $[MH]^+$. This also indicates that the peak contained multiple triacylglycerols. The acylium ions $[RCO + 74]^+$ at m/z 229, 257, 285, and 341 further confirmed the presence of capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), and stearic acid (C18:0) in the triacylglycerols at a retention time of 17.53 min. All compounds in this peak had an ACN of 32, and judging from their fatty acid moieties, they were confirmed to be triacylglycerols from coconut oil. The peak did not contain triacylglycerols with acetic acid (C2:0), propionic acid (C3:0), or butyric acid (C4:0). Since all of Salatrim triacylglycerols with ACN less than 42 have at least one of the short-chain fatty acids (acetic, propionic, butyric acids), this further confirms that the peak did not come from Salatrim.

Salatrim in Foods As Determined by the SFE/HTCGC Method and the Assessment of the Methodology. A summary of the theoretical Salatrim levels and the quantitative results as determined by this approach are listed in Table 3. The *precision* of the method was established by the triplicate analyses of each sample. Each analysis included the supercritical fluid crude fat extraction and the consequent HTCGC analysis. Results show that the % CV for the 11 samples containing Salatrim ranged from less than 1 to 6.2% (Table 3). The *accuracy* of the method was calculated as the difference between the mean measured Salatrim content (x) and the theoretical value (u) and are found in Table 3. To determine precision, the total error (E) was used to express the accuracy of the method as described by Karns et al. (1991). The total error was calculated as follows:

$$E = |x - u| + 2.58\ \mathrm{SD} \qquad (7)$$

For each sample, the standard deviation (SD) was calculated from the triplicate analysis of the sample. Table 3 summarizes the precision, the accuracy, and the total error for each of the 12 samples analyzed. Further statistical analysis indicates that the correlation coefficient (R^2) between the means of 11 calculated values and their corresponding theoretical values was 0.99.

The method used in this study is an application of two established methodologies, a nontraditional crude fat extraction and an IUPAC method of fats and oil analysis. The precision and accuracy of this study was very close to that of previous study (Huang et al., 1995).

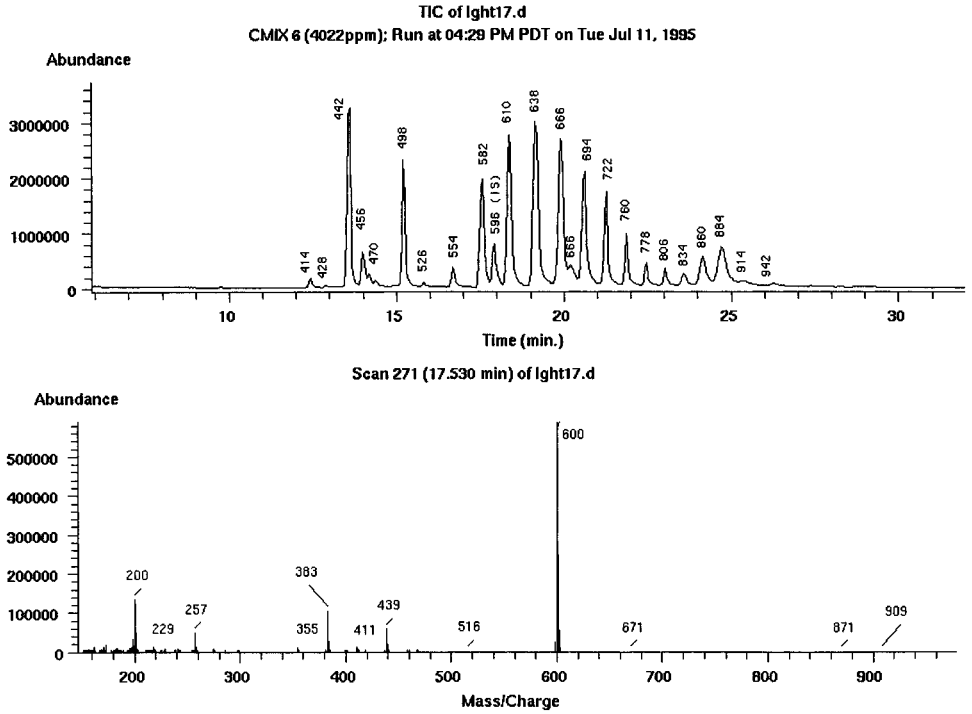

Figure 7. HTCGC-PCI mass spectrometry total ion chromatogram (TIC) of the crude fat isolated from the peanut cup sample 6 (top) and PCI spectrum of the component at retention time of 17.53 min (bottom). The molecular weight of each major component is indicated on the TIC. The peak retention time 17.53 min was confirmed to have ACN 32. The peak is a mixture of several triacylglycerols with the same ACN. These components were triacylglycerols with combinations of caprylic, capric, lauric, myristic, palmitic, and stearic acids.

Table 3. Theoretical Values of Salatrim and the Percent Salatrim As Determined by Analysis[a]

	sample											
	1	2	3	4	5	6	7	8	9	10	11	12
Salatrim type	none	4SO	23SO	23/4SO[b]	23/4SO[b]	23/4SO[b]	23/4SO[b]	23/4SO[b]	23/4SO[b]	23/4SO[b]	23/4SO[b]	23/4SO[b]
						Theoretical Values						
	0	*4.32*	*8.23*	*3.14*	*6.28*	*9.38*	*12.48*	*15.64*	*21.8*	*24.89*	*28.05*	*31.2*
						By Analysis						
run 1	0	3.92	7.63	3.12	5.62	9.23	12.13	14.76	21.48	25.2	28.2	31.9
run 2	0	3.88	7.81	3	5.76	8.92	11.26	15.21	20.94	25.5	28.31	32.4
run 3	0	3.95	7.37	2.81	5.73	8.17	12.4	15.4	22.5	25.4	28.2	31.5
average	*0*	*3.92*	*7.60*	*2.98*	*5.70*	*8.77*	*11.93*	*15.12*	*21.64*	*25.37*	*28.24*	*31.93*
SD	0	0.04	0.22	0.16	0.07	0.55	0.60	0.33	0.79	0.15	0.06	0.45
% CV	na[e]	0.9	2.9	5.3	1.3	6.2	5.0	2.2	3.7	0.6	0.2	1.4
accuracy[c] (%)	0	−0.4	−0.63	−0.16	−0.58	−0.61	−0.55	−0.52	−0.16	0.48	0.19	0.73
total error[d]	0	0.503	1.198	0.573	0.761	1.579	2.098	1.371	2.198	0.867	0.345	1.891

[a] All data were weight percents of the corresponding sample. [b] Salatrim 23/4SO was a combination of Salatrim 23SO and Salatrim 4SO. The ratio of Salatrim 23SO to Salatrim 4SO varied with the individual formulations as described in the Experimental Section. [c] Accuracy is expressed as the difference between the calculated value average and the theoretical value. [d] Total error (E) $E = |x - u| + 2.58SD$ (*Pharm. Res.* **1991**, *8*, 421−426). [e] Not available.

CONCLUSIONS

Salatrim contains ~95% by weight of triacylglycerols whose ACNs range from 20 to 42. ACN profiling by HTCGC can be used to separate Salatrim from the majority of common vegetable oils whose ACNs are larger than 48. Some fats and oils, such as butter fat and coconut oil, contain triacylglycerols with ACN less than 42. However, butter fat and coconut oil have some unique ACNs that are absent in Salatrim, e.g., ACN 46 from butter fat and ACN 32 and 34 from coconut oil. These unique ACNs can be used to estimate the composition of Salatrim and other fats. As a result, crude fats from food items can be analyzed and the levels of Salatrim and other fats can be determined. In a previous report, Huang et al. (1995) demonstrated that Salatrim can be accurately determined in foods containing Salatrim, butter fat, and other common vegetable oils. In this continuation of the study, we describe a method that can determine Salatrim in foods made with Salatrim, coconut oil, and other common vegetable oils with accuracy and precision.

A total of 12 samples made with Salatrims and coconut oil at different concentrations and common vegetable oils were used in this study. The method used SFE to obtain a crude fat extract with unaltered triacylglycerols. The crude fat was then subjected to short nonpolar column HTCGC analysis to determine the level of Salatrim. The results obtained from this study show the following: (1) The crude fat obtained by supercritical fluid carbon dioxide extraction was comparable to total fat determined by the AOAC Soxhlet extraction method without alteration of the triacylglycerol structures, (2) the crude fat was separated according to ACNs which were confirmed by the HTCGC-MS;

and (3) levels of Salatrim determined by this method were close to the theoretical values as calculated from the formulations with a correlation coefficient of 0.99.

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