Quantification of Generic SALATRIM Material in Foods by the Combination of Nontraditional Crude Fat Extraction and Short Nonpolar Column High-Temperature Capillary Gas Chromatography

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This publication describes the methodology used in the quantification of generic SALATRIM in foods. SALATRIM is a family of structured triacylglycerols developed by the Nabisco Foods Group. It is an interesterification product of triacetin, tripropionin, tributyrin, and hydrogenated vegetable oils. Approximately 95% by weight of the SALATRIM components are either triacylglycerol species with two short-chain fatty acids and one long-chain fatty acid or triacylglcerol species with two long-chain fatty acids and one short-chain fatty acid. To develop a method to quantify generic SALATRIM material in foods, 11 food items (baked goods, confectionery products, and ice cream product) made with several SALATRIM materials at different concentrations, common vegetable oil, dairy fat, and cocoa butter as their main fat sources were evaluated. This publication reports on the extraction of crude fat from the food products by supercritical fluid carbon dioxide or 2-propanol–hexane column extraction method (WCR method) without altering their intact triacylglycerol structures and on the separation of SALATRIM from other lipid sources and quantification by high-temperature capillary gas chromatography with a short SIM-DIST fused silica capillary column GC analysis.

Keywords: *SALATRIM; triacylglycerol; food; ACN (acyl carbon number); SFE (supercritical fluid extraction); HTCGC (high-temperature capillary gas chromatography); HTCGC–PCI-MS (high-temperature capillary gas chromatography with positive chemical ionization mass spectrometry)*

INTRODUCTION

SALATRIM is a family of low-calorie triacylglycerols developed by the Nabisco Foods Group. The materials were prepared by the interesterification of hydrogenated vegetable oils with appropriate short-chain triacylglycerols followed by distillation as described by Klemann et al. (1994). The final product is a complex mixture of triacylglycerols with various short- and long-chain fatty acids esterified onto the glycerol backbone and diacylglycerols with one short-chain and one long-chain fatty acid. SALATRIM contains 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). To quantify SALATRIM material in foods, Huang et al. (1994b) used supercritical fluid carbon dioxide to quantitatively extract crude fat from foods containing SALATRIM 23CA*, followed by HPLC analysis with an evaporative light-scattering detector to quantify SALATRIM 23CA in foods. The critical steps of such analysis depend on (1) the exhaustive extraction of crude fat without altering intact triacylglycerol structures, (2) the effective resolution of SALATRIM triacylglycerols from triacylglycerols of other fat sources, and (3) the identification and correlation of individual triacylglycerols to their corresponding fat source. [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).]

For exhaustive crude fat extraction, the traditional procedure often involves acid/alkaline hydrolysis, multistep extraction procedures, and Soxhlet extraction. These procedures are prone to the formation of artifacts, such as hydrolysis of triacylglycerols and *cis–trans* isomerization of polyunsaturated fatty acids. Several alternative fat extraction methods that offer milder extraction conditions with minimum artifact formation have been reported. One approach gaining in popularity is supercritical fluid extraction (SFE). The advantages of SFE over traditional methods of crude fat extraction have been discussed in numerous publications (Wright et al., 1988; Friedrich et al., 1982). Recently, Huang et al. (1994b) extracted SALATRIM 23CA without altering the triacylglycerol structures by using supercritical fluid extraction. Another popular approach involves the modification of extraction solvent to reduce the interfering materials for subsequent analysis. For example, Hara and Radin (1978) first proposed the use of a hexane–2-propanol (3:2 v/v) (HIP) mixture to extract lipids from biological tissues. The resulting crude fat extract was reported to have less non-lipid material than fat extracts obtained with the traditional solvents. Wolff and Castera-Rossignol (1987) further modified the method by combining the HIP mixture with filtration through a Celite–sodium sulfate column to extract fat from processed cheeses (WCR method). Wolff and Fabien (1989) reported that the fat extracted by the WCR method was practically free of dairy protein and did not change the fatty acid structures.

High-performance liquid chromatography (HPLC) is the most popular chromatographic technique for the

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separation of lipid material into species or groups. Its excellent resolving power has been known for some time (Smith et al., 1980; El-Hamdy and Perkins, 1981). The application of reversed phase HPLC (RPHPLC) for the quantification of various fats and oils and SALATRIM material has been reported by Palmer and Palmer (1989), Stolyhwo et al. (1985), and Huang et al. (1994b). Among all chromatographic techniques, gas–liquid chromatography (GLC) is the most efficient choice for lipid separation and quantitation (Kuksis and Myher, 1987). Longer polar columns, such as the 65% phenylmethylsilicone capillary column, separate triacylglycerols not only on the basis of total acyl carbon number but also on the basis of the number of double bonds. This type of polar column offers excellent resolution of triacylglycerol species. On the other hand, short nonpolar columns, such as an OV-1 column, separate triacylglycerols on the basis of total acyl carbon number. This type of column offers greater recovery and better quantification for triacylglycerols. 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 using a short fused silica capillary column with a chemically bonded nonpolar stationary phase. High-temperature gas chromatography (HTGC) using short packed columns and a capillary column has been recommended to provide quantitative profiles of fats and oils by IUPAC (1985). The analysis of intact lipid materials by HTCGC has been greatly enhanced by coupling with mass spectrometry. The online coupling of packed column GC–MS with ammonia chemical ionization was first applied in the identification of triacylglycerols by Murata (1977) and Murata and Takahashi (1977) in 1977. Since then, the application of GC–mass spectrometry in lipid characterization with either packed or capillary column has been reported by many researchers. For example, Oshima et al. (1989) used a phenylmethylsilicone stationary phase column and GC–MS with selective ion monitoring to analyze the molecular species of vegetable oil triacylglycerols. More recently, Huang et al. (1994a) used a 65% phenylmethylsilicone capillary column GC combined with ammonia chemical ionization mass spectrometry to identify various SALATRIM triacylglycerols.

The analysis of total fats and oils in food products is extremely important in the food industry. A desirable chemical evaluation not only determines the fat content but also determines the contents of individual fat sources. In a previous study, with reference SALATRIM 23CA material, Huang et al. (1994b) used the combination of SFE, HPLC, and particle beam LC–mass spectrometry to determine the SALATRIM 23CA* contents in foods. In this study, without using reference SALATRIM material, we report on (1) the use of nontraditional crude fat extraction methods to perform a quantitative extraction of crude fat that contains various SALATRIM materials from foods; (2) the use of a nonpolar short column to separate the crude fat triacylglycerols according to their acyl carbon numbers; (3) the use of HTCGC with positive chemical ionization mass spectrometry to confirm the SALATRIM components; and (4) the quantification of generic SALATRIM material in foods by quantifying 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.

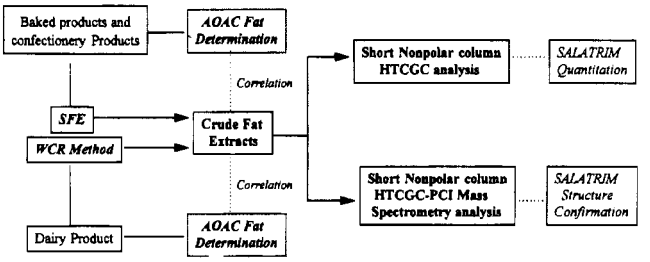

Figure 1. Overview of the steps for the quantification of SALATRIM in foods.

EXPERIMENTAL PROCEDURES

An outlined experimental procedure used in this study is shown in Figure 1.

Materials. Twelve food products (seven cookie samples, four chocolate-coated cookie samples, and one chocolate frozen dessert sample) were evaluated. The samples were manufactured with one or more of the following oils as their fat source: SALATRIM 4SO, SALATRIM 23SO, SALATRIM 24SO, SALATRIM 24SO/4SO, hydrogenated soybean oil, butter fat, and cocoa butter. SALATRIMs 4SO, 23SO, 24SO, and 24SO/4SO are members of the SALATRIM family of triacylglycerols developed by the Nabisco Foods Group. All SALATRIM materials were obtained from the Nabisco Foods Group Special Project Group, E. Hanover, NJ. Hydrogenated soybean oil and butter fat were obtained from the Nabisco Biscuit Product Development Group, E. Hanover, NJ. The seven cookie samples and four chocolate-coated cookies were manufactured at the Nabisco Biscuit Development Center, Fair Lawn, NJ. The frozen dessert sample (chocolate ice cream) was made at the food processing laboratory, Department of Food Science, Cornell University, Ithaca, NY. Descriptions of the finished products follow.

(1) Cookie Samples 1–7. These seven cookie samples were formulated to contain 20% fat from different sources. Other ingredients included Climax flour, FG sugar, brown sugar, high fructose corn syrup, whey, ammonium bicarbonate, diammonium phosphate, salt, soda, vanilla extract, and water. The composition of fat used in each of the cookie samples was as follows: sample 1, 4% SALATRIM 24SO, 16% hydrogenated soybean oil; sample 2, 10% SALATRIM 24SO, 10% hydrogenated soybean oil; sample 3, 16% SALATRIM 24SO, 4% hydrogenated soybean oil; sample 4, 4% SALATRIM 23SO, 16% hydrogenated soybean oil; sample 5, 10% SALATRIM 23SO, 10% hydrogenated soybean oil; sample 6, 16% SALATRIM 23SO, 4% hydrogenated soybean oil; sample 7, 20% hydrogenated soybean oil. Sample 7 was used in total fat determination but was not used in the SALATRIM content analysis.

(2) Chocolate-Coated Cookies 8 and 9. The inner cookie was the same as cookie sample 7. The compound coating of sample 8 was made with 30% SALATRIM 24SO/4SO, 12% cocoa powder, 0.4% lecithin, and 58% sugar/nonfat dry milk. The compound coating of sample 9 was made with 30% SALATRIM 23SO, 12% cocoa powder, 0.4% lecithin, and 58% sugar/nonfat dry milk. The cocoa powder contained 10–12% cocoa butter. The weight ratio between the inner cookie and compound coating was 52.12% to 47.88% for sample 8. Sample 9 had 57.48% by weight of cookie and 42.52% by weight of compound coating.

(3) Chocolate-Coated Butter Cookies 10 and 11. The butter cookie was made with 23.3% butter as its main fat ingredient. Other ingredients included flour, sugar, milk powder, egg powder, salt, and chemical leavening agent. The compound coating for sample 10 was the same as that used in sample 8. The compound coating for sample 11 was the same as that used in sample 9. The weight percentages for the butter cookie center and outer compound coating in sample 10 were 38.12% and 61.88%, respectively. Sample 11 had 36.1% by weight of butter cookie and 63.9% by weight of compound coating.

(4) Frozen Dessert Sample (Chocolate Ice Cream Sample 12). The frozen dessert sample was made with 10% SALATRIM 4SO, 64.9% skim milk, 5.4% nonfat dry milk, 13% granulated

sugar, 3% dry corn syrup solids, 0.14% Dricol 200 as stabilizer, and 3.5% cocoa powder.

Fat Extraction and Determination of Crude Fat Content. (A) Fat Content Determined According to AOAC Standard Procedures. The fat contents of the cookie samples (1–7) and chocolate-coated samples (8–11) were determined according to AOAC Acid Hydrolysis Method 14.019 (AOAC, 1984a). The fat content of the frozen dessert sample (sample 12) was determined according to AOAC Roese-Gottlieb Method 16.316 (AOAC, 1984b). The fat content was expressed in weight percentage of the corresponding food item.

(B) SFE Fat Extraction Procedure. The cookie samples and chocolate-coated cookie samples were used directly for supercritical fluid extraction without further treatment. Each sample was ground to a fine paste or fine powder. A 0.5 g weight of each sample was used for the 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 then 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 percentage of fat in the corresponding sample. Supercritical carbon dioxide extraction was performed with a Hewlett-Packard 7680T extraction module and the conditions were as follows.

Extraction Conditions. Approximately 0.5 g of sample was placed in 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 × 1cm o.d.) was also 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 extraction fluid. CO_2 was maintained at a density of 0.8 g/mL, pressure at 365 bar, and flow rate of 4 mL/min. After density and temperature were reached, the sample was soaked in the SF CO_2 for an equilibration time of 2 min without CO_2 flow through the chamber. After the equilibration time ended, the CO_2 fluid flowed through the sample thimble and carried 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 rinse solvent. The nozzle temperature and trap temperature at the rinsing stage were both kept at 30 °C.

(C) Fat Content Determined by 2-Propanol-Hexane Column Method (WCR Method). The fat content of the frozen dessert was determined using the method described by Wolff and Fabien (1989).

Chromatographic Column. A glass column (10.5 mm i.d. × 300 mm length) with Teflon stopcock was used for the crude fat extraction. Approximately 3 g of anhydrous sodium sulfate (reagent grade, Aldrich Chemical Co.) was added to form the bottom layer of the chromatographic column. A preweighed round-bottom flask was placed under the column as receiving flask.

Sample Preparation. Approximately 3 g of Celite (J. T. Baker Chemical) and 2 g of frozen dessert sample were placed into a 50 mL beaker. Twenty-five milliliters of hexane/2-propanol mixture (3/2 v/v) was then added into the beaker. The sample, Celite, and solvent mixture were then mixed thoroughly.

Extraction of the Crude Fat. The sample mixture was transferred to the chromatographic column. Five milliliters of hexane/2-propanol (3/2 v/v) was used to rinse the beaker and transfer the residual sample onto the column. After the Teflon stopcock was opened, another 75 mL of hexane/2-propanol (3/2 v/v) was added into the column. The flow into the receiving flask was kept at approximately 2 mL/min.

Crude Fat Collection and Determination. At the end of elution, the receiving flask was removed and the solvent was removed with a rotary evaporator kept at a temperature of 55–60 °C and under a vacuum of 550–660 mmHg. Any residual solvent was further removed in an air oven kept at 60 °C for 30 min. The dried flask was then kept in a desiccator and allowed to cool to room temperature. The weight of the dried flask and its content of crude fat was then determined. The weight of crude fat was the weight difference between the dried flask with extracted material and the preweighed empty flask. The fat content (C_{fat}) was expressed as the weight percentage of crude fat in the frozen dessert.

(D) Statistical Analysis of Various Crude Fat Contents. The standard deviations, percent coefficients of variation (CV%), and the correlation coefficient (R^2) between results from the AOAC and nontraditional methods were calculated according to the AOAC guide *Use of Statistics to Develop and Evaluate Analytical Method* (Wernimont, 1985).

Analysis of Standard Solution and SALATRIM Materials by the Chrompack SIM-DIST CB Capillary Column HTCGC. *Solvent.* Both undecane and toluene were of spectrometric grade and were obtained from Aldrich Chemical Co., St. Louis, MO.

Triglyceride Standard. Thirteen triacylglycerol standards (tricaproin, triheptanoin, tricaprylin, trinonanoin, tricaprin, triundecanoin, trilaurin, tritridecanoin, trimyristin, tripentadecanoin, tripalmitin, triheptadecanoin, and 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 Butter Fat Solutions. (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 butter fat solution contained about 3000 mg/L of butter fat in stock solution.

Extracted Crude Fat Solutions for SALATRIM Quantification. Each test solution contained approximately 3000 mg/L of the extracted crude fat material in stock solution. Crude fat extracts were obtained from the SFE and WCR crude fat extraction of the 12 food samples. Each test solution contained 100 ppm (μg/mL) of triundecanoin as internal standard.

Analysis of Triacylglycerol Standard Solution, Butter Fat Solution, and Extracted Crude Fat Solution by HTCGC with Chrompack SIM-DIST CB Capillary Column. Analyses of triacylglycerol standard solutions, butter fat solution, and crude fat solutions were performed as follows: The crude fat solution was first filtered through an Acrodisc CR PTFE syringe filter (Scientific Resources Inc., Eatontown, NJ) before the HTCGC analysis. The analyses were performed on a Hewlett-Packard 5890 Series II GC system. The system was equipped with a flame ionization detector, pressure programmable on-column injector, HP 7673 autosampler (with nanoliter adapter and 5 μL syringe), and HP DOS ChemStation data system (Hewlett-Packard Co., Palo Alto, CA). The separation was accomplished with a Chrompack SIM-DIST CB fused silica column (5.0 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 350 °C at 10 °C/min and held at 350 °C for 2 min. 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. 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.

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 (Hewlett-Packard) was used for the analysis. The

Hewlett-Packard 5890 GC was equipped with a pressure programmable on-column injector and a HP 7673 autosampler.

Gas Chromatography. A Chrompack SIM-DIST CB fused silica column (10 m × 0.32 mm i.d., 0.1 μm film thickness, Chrompack) 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). The oven temperature was ramped from 50 to 150 °C at 18 °C/min, 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 10 psi (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 PPG (polypropylene glycol, Hewlett-Packard). A scan range of m/z 150–1000 was used. A Hewlett-Packard UNIX ChemStation (Hewlett-Packard) software package was used for data acquisition and data processing.

Determination of the Relative Response Factors of Standard Triacylglycerols and the Establishment of 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 ACNs were used. They were tricaproin, triheptanoin, tricaprylin, trinonanoin, tricaprin, triundecanoin, trilaurin, tritridecanoin, trimyristin, tripentadecanoin, tripalmitin, triheptadecanoin, and tristearin. Triundecanoin was used as internal standard. The relative response factor to triundecanoin (RF_i) for each of the standard triacylglycerols of ACNi at retention time t was determined by the 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 Relative Response Factors of Triacylglycerol Components and Their Weight Percents in the Crude Fat Extract and in the Butter Fat Solution. The relative response factors and the weight percentage of each triacylglycerol in crude fat and in butter fat were obtained from the analyses of the crude fat solution and butter fat solution. The concentration (μg/mL) of each triacylglycerol of ACNi (C_i) in the solution was calculated by the 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 ACNi 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 percentage of each triacylglycerol with ACNi (WT_i) in the crude fat extract material or in the butter fat was calculated as

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

where C_T is the known concentration of crude fat material or butter fat in the test solution.

Determination of Generic SALATRIM Contents in Foods That Contain No Butter Fat (Samples 1–6, 8, 9, and 12) from the Analysis of Crude Fat Solution. The SALATRIM contents in food samples 1–6, 8, 9, and 12 were determined by the combined weight of triacylglycerols with ACN 20 to ACN 42 as indicated in

$$WT_{SAL} = \sum_{i=20}^{42} WT_i \times 100/95 \quad (4)$$

$$C_{SAL} = WT_{SAL}C_{fat} \times 100 \quad (5)$$

where C_{SAL} is the weight percentage of SALATRIM in foods, WT_{SAL} is the weight percentage of SALATRIM in the extracted crude fat, and C_{fat} is the weight percentage of crude fat in foods as determined by the SFE or WCR extraction.

Determination of Generic SALATRIM Contents in Foods That Contain Butter Fat (Samples 10 and 11). *Determination of the Ratio (R_{B46}) between the Combined Weight of Butter Triacylglycerols with ACN 20–42 and the Weight of Butter Triacylglycerols of ACN 46 from the Analysis of Reference Butter Fat Solution.* From the HTCGC analysis of the butter fat solution, the ratio (R_{B46}) between the combined weight of butter fat triacylglycerols with ACN 20–42 ($WTB_{ACN20-42}$) and the weight of butter fat triacylglycerols at ACN 46 (WTB_{ACN46}) was calculated as follows:

$$R_{B46} = WTB_{ACN20-42}/WTB_{ACN46} = \sum_{i=20}^{42} WT_i/WT_{46} \quad (6)$$

Determination of SALATRIM Contents in Food Products with Butter as Part of Their Fat Source from the Analysis of Crude Fat Solution. The SALATRIM contents in the chocolate-coated butter cookies were calculated from the HTCGC analysis of the corresponding crude fat sample. The SALATRIM weight was determined by subtracting the total weight of butter fat components with ACN 20–42 from the total weight of components with ACN 20–42. The equations are

$$WT_{SAL} = (WT_{ACN20-42} - WTB_{ACN20-42}) \times 100/95$$
$$= (\sum_{i=20}^{42} WT_i - WT_{46} \times R_{B46}) \times 1.053 \quad (7)$$

$$C_{SAL} = WT_{SAL}C_{fat} \times 100 \quad (8)$$

where $WT_{ACN20-42}$ is the total weight of triacylglycerol with ACN from 20 to ACN 42 and $WTB_{ACN20-42}$ is the total weight of butter fat triacylglycerols having ACN from 20 to 42. R_{B46} was obtained from butter fat analysis and calculated from eq 6; C_{SAL} is the weight percentage of SALATRIM in foods, WT_{SAL} is the weight percentage of SALATRIM in the extracted crude fat, and C_{fat} is the weight percentage of crude fat in foods as determined by the SFE or WCR extraction.

Statistical Analysis of SALATRIM Contents in Foods. The standard deviations, percent coefficients of variation (CV%), and correlation coefficients (R^2) between results from the calculated values and the theoretical values were calculated according to the AOAC guide *Use of Statistics to Develop and Evaluate Analytical Method* (Wernimont, 1985). Total error was calculated according to the publication of Karns et al. (1991).

RESULTS AND DISCUSSION

Total Fat Content Determined According to AOAC Standard Methods and Alternative Methods. Huang et al. (1994a) published the exhaustive extraction of crude fat from sandwich cookies, bonbons, and ice cream by supercritical fluid extraction and showed that the complete recovery of intact triacylglycerols from SALATRIM 23CA can be achieved by SFE.

Table 1. Crude Fat Contents by AOAC Method and Alternative Method

		by AOAC standard method[a]						by alternative method[a]						
		run 1	run 2	run 3	av	SD	CV%	run 1	run 2	run 3	run 4	av	SD	CV%
	baked product													
1	cookie	22.4	22.8	21.9	**22.37**	0.45	**2.02**	23.16	22.34	23.15	22.63	**22.82**	0.40	**1.17**
2	cookie	22.6	22.7	22.6	**22.63**	0.06	**0.26**	22.03	22.62	22.35	21.89	**22.22**	0.33	**1.47**
3	cookie	22.4	22.1	22.5	**22.33**	0.21	**0.93**	23.98	24.55	23.91	22.85	**23.82**	0.71	**2.98**
4	cookie	22.3	22.2	22.6	**22.37**	0.21	**0.93**	22.81	21.87	22.66	23.64	**22.75**	0.73	**3.19**
5	cookie	21.7	22.2	22.6	**22.17**	0.45	**2.03**	22.08	21.84	21.40	21.51	**21.71**	0.31	**1.43**
6	cookie	22.2	22.4	21.9	**22.17**	0.25	**1.14**	21.95	22.00	21.94	22.37	**22.07**	0.21	**0.93**
7	cookie	23.5	23.4	23.5	**23.47**	0.06	0.25	21.97	21.78	21.85	21.58	21.80	0.16	0.75
	confectionary product													
	nondairy type													
8	chocolate-coated cookie	27.7	27.5	28	**27.73**	0.25	**0.91**	27.28	25.53	27.47	27.19	**26.87**	0.90	**3.35**
9	chocolate-coated cookie	27.5	29.1	28	**28.20**	0.82	**2.90**	28.87	29.75	28.70	28.41	**28.93**	0.58	**1.99**
	dairy type													
10	chocolate-coated butter cookie	28.4	28.6	28.3	**28.43**	0.15	**0.54**	29.20	28.64	27.47	28.33	**28.41**	0.72	**2.54**
11	chocolate-coated butter cookie	28.5	28.4	27.2	**28.03**	0.72	**2.58**	27.35	26.90	27.21	27.54	**27.25**	0.27	**0.99**
	frozen dessert													
12	chocolate ice cream	10.9	10.8	11	**10.90**	0.10	**0.92**	11.82	11.18	11.38	11.46	**11.46**	0.27	**2.33**

[a] All data expressed as weight percentage except for SD and CV.

In this study, the crude fat was extracted from the ice cream sample by SFE after lyophilization. Wolff and Castera-Rossignol (1987) proposed combining hexane–2-propanol (3:2 v/v) extraction with filtration on a Celite–sodium sulfate column (WCR method) to extract fat from processed cheeses. Wolff and Fabien (1989) showed that the crude fat isolated from dairy products by this method did not alter the fatty acid structure. The WCR method did offer significant advantages over the SFE method because the method does not require the lyophilization of dairy products (such as ice cream product) prior to fat extraction. In this study, the crude fat in frozen dessert (chocolate ice cream) was extracted following the method described by Wolff and Fabien (1989), and the crude fat from the baked products (cookies) and confectionery products (chocolate-coated cookies) was extracted following the supercritical fluid extraction method of Huang et al. (1994).

To determine the time and amount of supercritical fluid CO_2 needed to perform an exhaustive extraction for baked goods and confectionery products, each sample was subjected to a series of extraction cycles as described in the previous study (Huang et al., 1994b). An extraction time of 30 min was determined to be sufficient for all samples. For the frozen dessert sample, to determine the amount of HIP solution needed for the exhaustive extraction by the WCR method, samples were subjected to a series of repeat extractions with 10 mL of HIP solvent in each extraction. At the end of each extraction, the crude fat collected was weighed and the weight increase was recorded. Extraction was determined to be complete when no weight gain was observed between consecutive extractions. An HIP volume of 90 mL was determined to be sufficient for the complete extraction of crude fat from the frozen dessert sample. A total of 105 mL of HIP mixture was used in this study.

The fat contents of each sample as determined by the AOAC method, SFE method, and WCR method are shown in Table 1. The percent recovery of crude fat from the baked goods and from the confectionery products by SFE ranged from 93% to 106% as compared to that of the AOAC acid hydrolysis method. The percent recovery of crude fat from the frozen dessert sample by the WCR method was 105% as compared to that of the AOAC Roese-Gottlieb method. The results also show the precision of the SFE method for baked and confectionery products; the coefficient of variation (CV%) ranged from less than 1% to 3.2%. The CV of the WCR method for the frozen dessert fat content was 2.33%. In comparison, the CV for the AOAC method ranged from 0.25% to 2.9%. Further statistical analysis of fat contents obtained by the alternative method (SFE and WCR method) and the traditional method (AOAC methods) showed that the correlation coefficient between two methods was 0.98. These data confirmed that the results obtained by both SFE and WCR methods were comparable to that of the AOAC method.

Triacylglycerol Separation and Quantification Consideration for SALATRIM Material. (A) The Analysis of the Crude Fat Extracts According to Their Acyl Carbon Number by Short Nonpolar Column. High-temperature gas chromatography (HTGC) using short nonpolar packed columns has been used to provide quantitative profiles of fats and oils (IUPAC, 1985). In the IUPAC method, a linear response factor was applied over the entire concentration range to each ACN. Mares and Husek (1985) also 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 sample amount analyzed and the carrier gas flow rate. In this study, a similar evaluation was conducted to evaluate the recovery of triacylglycerols with ACN from 18 to 54 on a 5 m SIM-DIST column. The results indicated that the response factors for triglycerides with ACN 18–54 are independent of quantities of injection for triacylglycerol in the range of 8 ng to over 2000 ng (per peak). The response of the flame ionization detector (FID) for triacylglycerol concentration less than 8 ng per peak was no longer linear. The recovery of triacylglycerol model compounds (ACN 18–54) analyzed by the short nonpolar Chrompack SIM-DIST CB column does not decrease with an increase in acyl carbon number (up to ACN 54). The short nonpolar column GC analysis follows the approach of the IUPAC method and is well suited for the quantification of SALATRIM triacylglycerols for the following reasons: (1) the SALATRIM triacylglycerols are essentially saturated triacylglycerols with ACN from 20 to 42 and (2) the recovery of triacylglycerol is independent of the sample amount range (from 8 ng to over 2000 ng per component), which covers the sample concentration range used in this study.

(B) Separation of SALATRIM Triacylglycerols from Other Fats by Short Nonpolar Column. Generic SALATRIM material contains mainly triacyl-

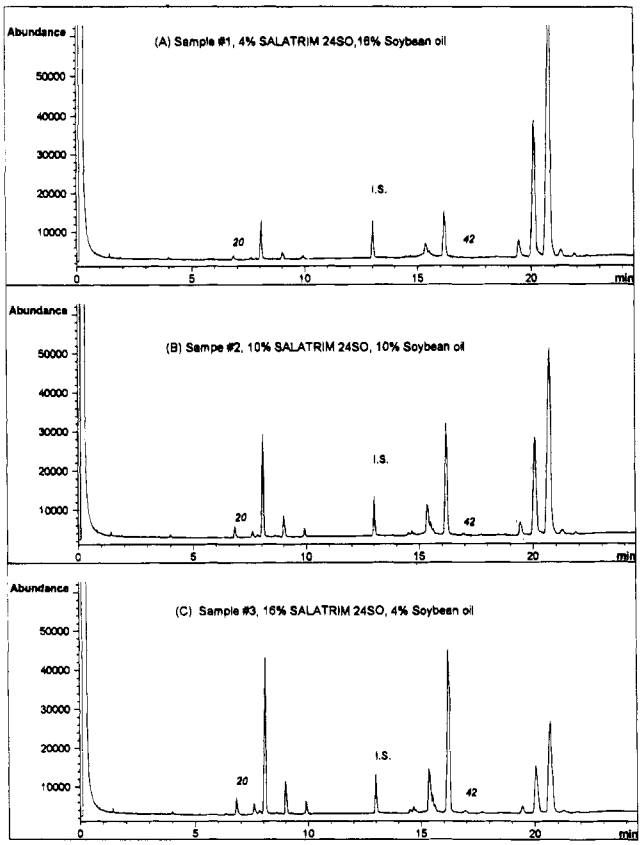

Figure 2. Comparative HTCGC profiles of the crude fat extracts obtained from (A) cookie sample 1, (B) cookie sample 2, and (C) cookie sample 3. Their fat compositions are listed in Table 2. Peak at RT = 13.1 min is internal standard triundecanoin. Triacylglycerols with ACN less than 42 are triacylglycerols originated from SALATRIM 24SO. Triacylglycerols with ACN greater than 46 arde triacylglycerols of soybean oil. Numbers indicated in the chromatograms are ACNs.

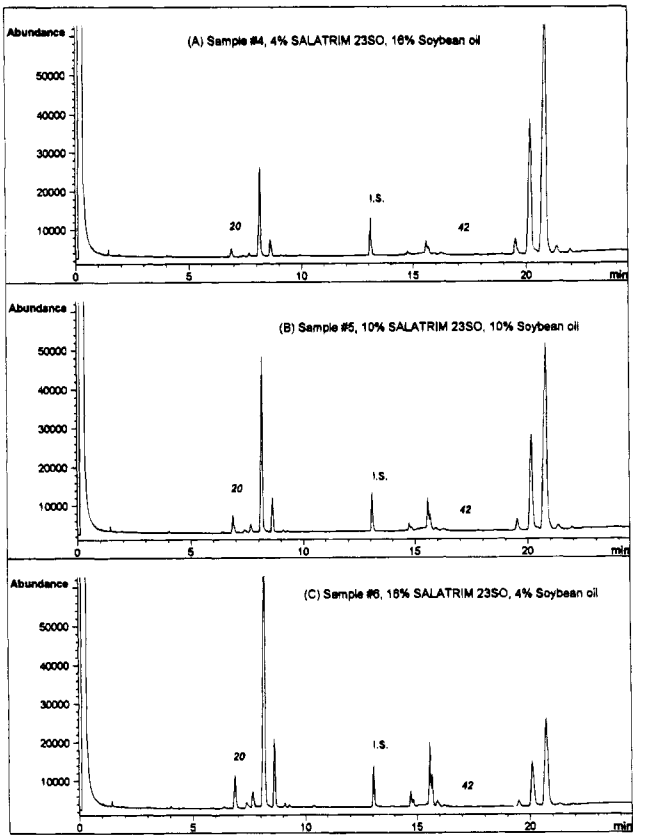

Figure 3. Comparative HTCGC profiles of the crude fat extracts obtained from (A) cookie sample 4, (B) cookie sample 5, and (C) cookie sample 6. Their fat compositions are listed in Table 2. Peak at RT = 13.1 min is internal standard triundecanoin. Triacylglycerols with ACN less than 42 are triacylglycerols originated from SALATRIM 23SO. Triacylglycerols with ACN greater than 46 are triacylglycerols of soybean oil. Numbers indicated in the chromatograms are ACNs.

glycerols 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-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 any SALATRIM materials, the triacylglycerol species are practically all within range of ACN 20–42. Those species account for about 95% by weight of SALATRIM material.

The triacylglycerols of common vegetable oil (such as soybean oil, sunflower oil, corn oil, and canola oil) have ACN greater than 42. The majority of these triacylglycerols are between ACN 48 and 56. Other commonly used oils, for example, palm oil and cocoa butter, also have triacylglycerols with ACN from 48 to 56. These oils can be distinguished from the SALATRIM materials by differences in triacylglycerol acyl carbon number. For example, Figure 2 shows the HTCGC separation of SALATRIM 24SO from soybean oil in the crude fats of cookie samples 1–3. Samples 1–3 were cookies made with SALATRIM 24SO and soybean oil as their fat sources. Sample 1 had 4% SALATRIM 24SO and 16% soybean oil. Sample 2 had 10% SALATRIM 24SO and 10% soybean oil. Sample 3 had 16% SALATRIM 24SO and 4% soybean oil. Triacylglycerols with ACN 20–42 were components of SALATRIM 24SO, and components with ACN greater than 46 were triacylglycerols of soybean oil. The peak areas of the components with ACN greater than 46 decreased from sample 1 through sample 3 due to the decrease of soybean oil in samples 1–3. On the other hand, the increasing concentration of SALATRIM 24SO in samples 1–3 caused the increase of peak areas of triacylglycerols with ACN 20–42 as shown in Figure 2. Figure 3 shows the comparative HTCGC profiles of the crude fats obtained from cookie samples 4–6. These three samples used SALATRIM 23SO and soybean oil as the fat source. Figure 3 indicates the separation of triacylglycerols of SALATRIM 23SO (with ACN 20–42) and soybean oil (with ACN greater than 46). A wide variety of SALATRIMs, including SALATRIM 4SO, 24SO, 24/4SO, 23SO, were used in this study. The triacylglycerols in these SALATRIM materials are a representation of all major triacylglycerols in generic SALATRIM. These SALATRIM triacylglycerols were distinguishable from many fat sources by their ACN differences as indicated in Figures 2–4. Figure 4 shows the HTCGC separation of SALATRIM 24SO/4SO from soybean oil/cocoa butter (in sample 8 chocolate-coated cookie), the separation of SALATRIM 23SO from soybean oil/cocoa butter (in sample 9 chocolate-coated cookie), and the separation of SALATRIM 4SO from cocoa butter (in sample 12 chocolate ice cream).

Some commercial fats and oils, such as coconut oil and butter fat, have significant amounts of short and medium fatty acids in their fatty acid profiles. Some of their major components are triacylglycerols with ACN

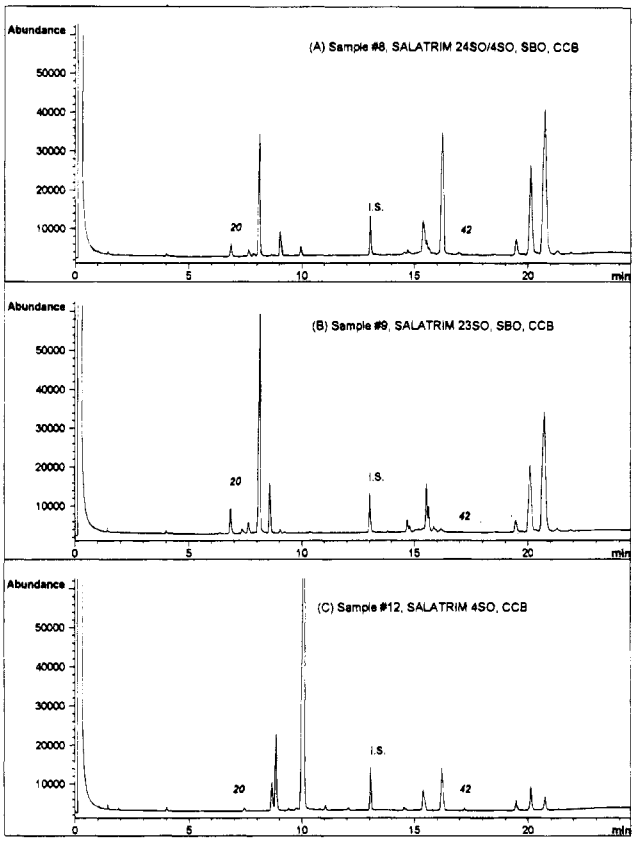

Figure 4. Comparative HTCGC profiles of the crude fat extracts obtained from (A) chocolate-coated cookie sample 8 made with SALATRIM 24SO/4SO, soybean oil, and cocoa butter, (B) chocolate-coated cookie sample 9 made with SALATRIM 23SO, soybean oil, and cocoa butter, and (C) chocolate ice cream made with SALATRIM 4SO and cocoa butter. Their fat compositions are listed in Table 2. Peak at RT = 13.1 min is internal standard triundecanoin. Triacylglycerols with ACN less than 42 are triacylglycerols originating from SALATRIM 24SO/4SO, SALATRIM 23SO, and SALATRIM 4SO in chromatograms A, B, and C, respectively. Triacylglycerols with ACN greater than 46 are combined triacylglycerols of soybean oil and cocoa butter. Numbers indicated in the chromatograms are ACNs.

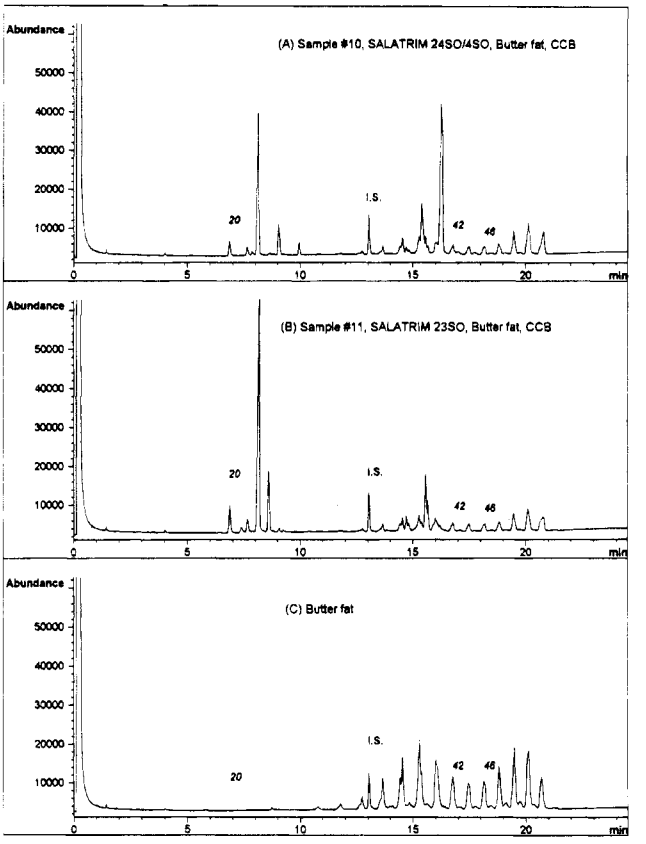

Figure 5. Comparative HTCGC profiles of the crude fat extracts obtained from (A) chocolate-coated butter cookie sample 10 made with SALATRIM 24/4SO, butter fat, and cocoa butter, (B) chocolate-coated butter cookie sample 11 made with SALATRIM 23SO, butter fat, and cocoa butter, and (C) reference butter fat. Their fat compositions are listed in Table 2. Peak at RT = 13.1 min is internal standard triundecanoin. Triacylglycerols with ACN less than 42 (components with retention time less than 17.00 min) are triacylglycerols originating from SALATRIM 24/4SO, SALATRIM 23SO, and butter fat in chromatograms A and B. Triacylglycerols at ACN 46 (retention time 18.2 min) originating from butter fat was used as reference peak to calculate the ratio (R_{B46}) of triacylglycerols 20–42 and that of ACN 46. Numbers indicated in the chromatograms are ACNs.

less than 42. For example, major fatty acids in butter include myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). Butter fat also has significant amounts of butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristoleic acid (C14:1), palmitoleic acid (C16:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidic acid (C20:0). Foods made with SALATRIM and butter fat have some triacylglycerols with ACN 20–42 originating from both SALATRIM and butter fat. For example, Figure 5 shows the comparison of the HTCGC profile of crude fats from chocolate-coated butter cookies (samples 10 and 11) with that of a reference butter fat. Sample 10 was made with SALATRIM 24SO/4SO, butter fat, and cocoa butter. Sample 11 was manufactured with SALATRIM 23SO, butter fat, and cocoa butter. Figure 5 shows that some of the SALATRIM triacylglycerols (ACN 20–42) were overlapped with triacylglycerols of butter fat. Butter fat has some unique triacylglycerols with ACNs that were practically absent from SALATRIM and cocoa butter. Some of these unique triacylglycerols are found at ACN 46. The triacylglycerol with ACN 46 was used to estimate the amount of butter fat triacylglycerols with ACN 20–42. The ratio (R_{B46}) between triacylglycerol with ACN 20–42 and triacylglycerols of ACN 46 was calculated from the analysis of the reference butter fat. Since triacylglycerols of ACN 46 in samples 10 and 11 were derived solely from the butter fat, the butter fat triacylglycerols with ACN 20–42 in the crude fat can be calculated from the R_{B46} ratio and the amount of triacylglycerols of ACN 46 in the crude fat extract. The total weight of triacylglycerols with ACN 20–42 in crude fat minus the amount of butter fat with ACN 20–42 is the amount of SALATRIM triacylglycerols. The R_{B46} ratio was calculated to be 9.65 for the reference butter fat used in this study.

In generic SALATRIM material, triacylglycerols with ACN 20–42 account for 95% by weight of the SALATRIM material. A correction factor of 1.053 (100/95) was used to reflect the true weight of SALATRIM in this study.

(C) Monitoring of SALATRIM Triacylglycerols by HTCGC–PCI Mass Spectrometry. The identification of triacylglycerol structures in SALATRIM material by the HTCGC–PCI-MS with ammonia reagent gas has been reported by Huang et al. (1994b). In positive [NH_3]CIMS spectrometry, a SALATRIM triacylglycerol with molecular weight M produces the pseudomolecular ion $[M + NH_4]^+$, fragment ions $[MH - RCOOH]^+$, and acylium ions $[RCO + 74]^+$. Fragment ion [MH –

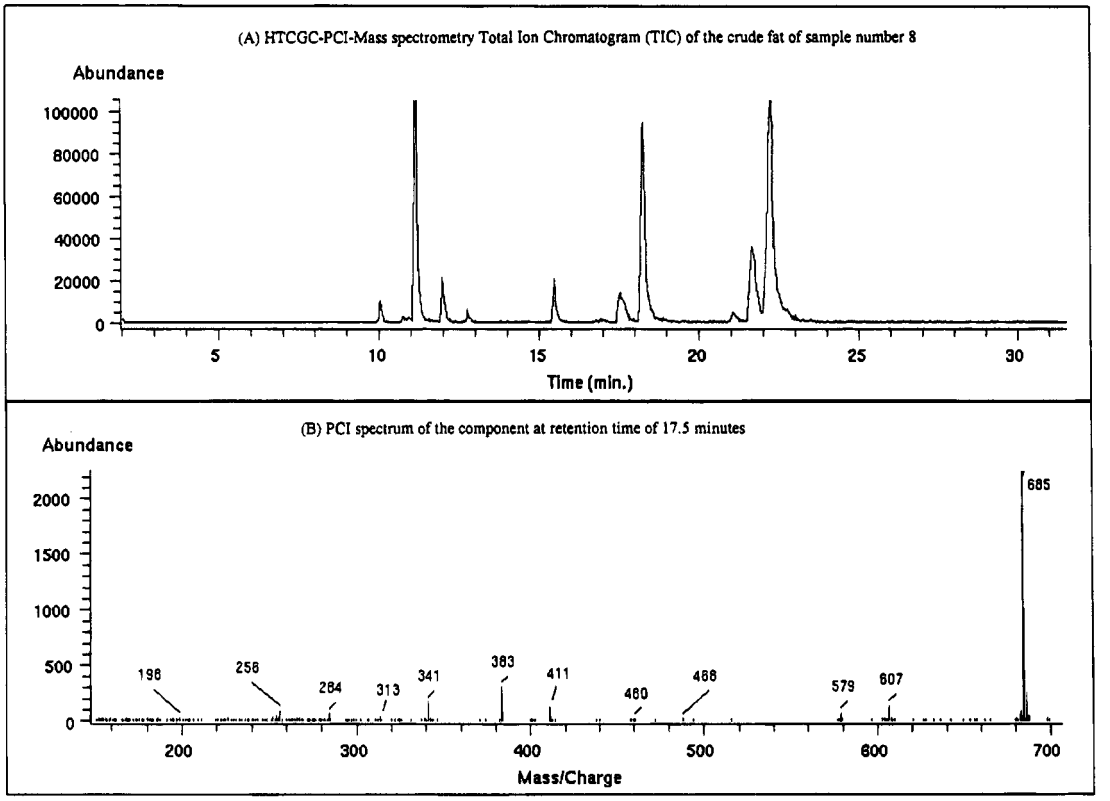

Figure 6. (A) HTCGC–PCI-mass spectrometry total ion chromatogram (TIC) of the crude fat of chocolate-coated sample number 8; (B) PCI spectrum of the component at retention time of 17.5 min. The peak was tentatively identified as triacylglycerols of diastearoylacetylglycerol and butyroylpalmitoylstearoylglycerol. The pseudomolecular ion $[MH + NH_4]^+$ of of 685 was a rollover number of exact mass of 684.6626. Both triacylglycerols have an ACN of 38.

$RCOOH]^+$ 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. Each peak may contain more than one triacylglycerol. HTCGC–mass spectrometry was used mainly to confirm the acyl carbon number rather than the positive identification of new triacylglycerol species. Figure 6 shows the positive chemical ionization total ion chromatogram of the crude fat isolated from chocolate-coated cookie sample 8 and PCI spectrum of the peak at retention time of 17.5 min. The component had a pseudomolecular ion $[M + NH_4]^+$ of 684. The molecular weight was determined to be 666, which confirmed that the component has an ACN of 38. The peak has fragment ions $[MH - RCOOH]^+$ of 607, 599, 411, and 383. These fragment ions indicated the losses of acetic acid, butyric acid, palmitic acid, and stearic acid from their corresponding protonated molecular ions $[MH]^+$. This also indicated that the peak contained multiple triacylglycerol species. The acylium ions $[RCO + 74]^+$ at 341 and 313 further confirmed the presence of stearic acid and palmitic acid in the triacylglycerols at retention time of 17.5 min. Triacylglycerol species in the peak were tentatively identified as distearoylacetylglycerol and butyroylpalmitoylstearoylglycerol. Both species were known triacylglycerol species of SALATRIM and were not triacylglycerols of soybean oil or cocoa butter. The composition of representative SALATRIM materials has been reported by Softly et al. (1994).

Assessment of SALATRIM Triacylglycerol Changes during Food Processing. SALATRIM triacylglycerol compositional changes, if any, occurring during food processing can be assessed by comparing the crude fat of the finished product with the original SALATRIM. The ACN profile as determined by the high-temperature capillary gas chromatography (HTCGC) can be used as one of the evaluations. Examples are illustrated in Figures 7 and 8.

SALATRIM 23SO was compared to the crude fat isolated from the cookies made with the same SALATRIM and other fat as shown in Figure 7. The ratios of major SALATRIM 23SO triacylglycerols with different ACN (ACN < 42) in the crude fat of cookies remain the same as those of the original SALATRIM. The ACN composition of SALATRIM 24SO and the crude fat isolated from the cookies made with the same SALATRIM is shown in Figure 8. The ratios of triacylglycerols with different ACNs less than 42 remain the same before and after processing. These observations indicate that (1) food processing, such as baking, does not change the SALATRIM ACN composition and (2) fat isolation methodology used in this study did not alter the ACN profile of the SALATRIM ingredients.

Calculated Results of SALATRIM Contents in Foods and Methodology Assessment. A summary of major fat sources as calculated from the formulations of the 12 samples analyzed is given in Table 2. The SALATRIM contents as determined by this approach are listed in Table 3. Several statistical analyses were used to evaluate the method: *precision* of the method, *accuracy* of the method, *total error* of the method, and

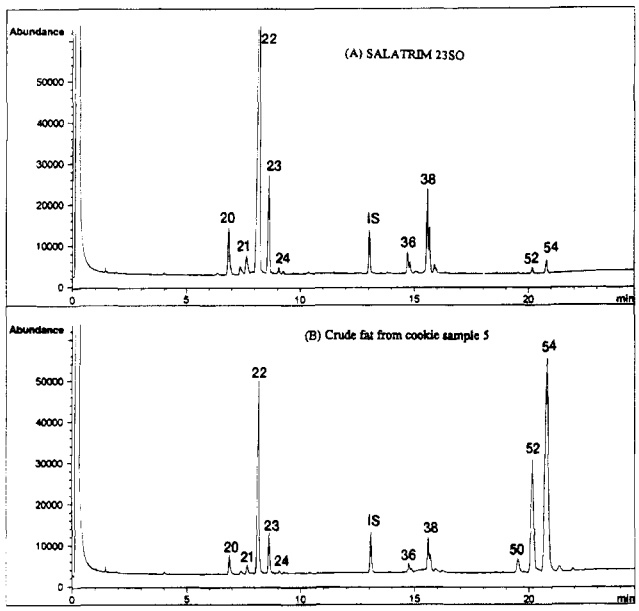

Figure 7. Comparative HTCGC profiles of SALATRIM 23SO (A) and crude fat isolated from cookie 5, which was made with 10% SALATRIM 23SO and 10% soybean oil. (B) In SALATRIM 23SO ACN profile (A), the ratios of triacylglycerols with ACN 20, 21, 22, 23, 24, 36, and 38 were 1.0, 0.23, 10.2, 2.0, 0.14, 0.64, and 2.5, respectively. In the crude fat isolated from the finished product (B), the ratio of the triacylglycerols with ACN 20, 21, 22, 23, 24, 36, and 38 were 1.0, 0.25, 10.0, 2.02, 0.12, 0.6, and 2.3, respectively. Triacylglycerols with ACN 20, 21, 22, 23, and 24 are SALATRIM triacylglycerols with two short-chain fatty acids (acetate and/or propionate) and one long-chain fatty acid. Triacylglycerols with ACN 36 and 38 are SALATRIM triacylglycerols with one short-chain fatty acid (acetate or propionate) and two long-chain fatty acids. SALATRIM 23SO triacylglycerols with ACN 50, 52, and 54 were mixed with soybean oil in the cookie. Internal standard (IS) was triundecanoin.

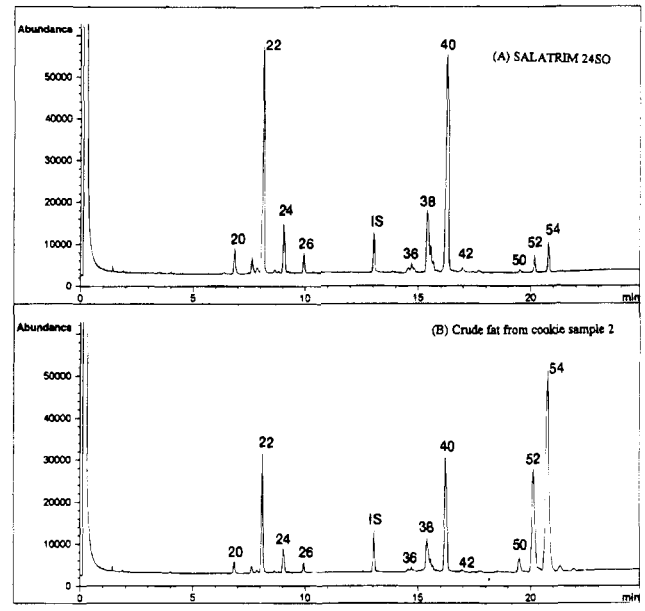

Figure 8. Comparative HTCGC profiles of SALATRIM 24SO (A) and crudefat isolated from cookie sample 2, which was made with 10% SALATRIM 24SO and 10% soybean oil (B). In SALATRIM 24SO (A), the ratios of triacylglycerols with ACN 20, 22, 24, 26, 36, 38, 40, and 42 were 1.0, 10.8, 2.2, 0.6, 0.7, 5.0, 14.1, and 0.24, respectively. In the crude fat isolated from the finished product (B), the ratio of the triacylglycerols with ACN 20, 22, 24, 26, 36, 38, 40, and 42 were 1.0, 10.0, 2.19, 0.7, 0.72, 6.0, 14.4, and 0.23, respectively. Triacylglycerols with ACN 20, 22, 24, and 26 are SALATRIM 24SO triacylglycerols with two short-chain fatty acids (acetate and/or butyrate) and one long-chain fatty acid. Triacylglycerols with ACN 36, 38, 40, and 42 are SALATRIM 24SO triacylglycerols with one short-chain fatty acid (acetate or butyrate) and two long-chain fatty acids. SALATRIM 24SO triacylglycerols with ACN 50, 52, and 54 were mixed with soybean oil in the cookie. Internal standard (IS) was triundecanoin.

correlation coefficient between the calculated values and the true values.

The *precision* of the method was established by the quadruple analyses of each sample. Each analysis included the nontraditional extraction (either by SFE or by WCR method) and the consequent HTCGC analysis. The results showed that the percent coefficient of variation (CV%) for the 11 samples ranged from less than 1% to 6.5% (Table 3). The *accuracy* of the method was obtained as the difference between the mean measured SALATRIM content (x) and the true value (u). The true values were theoretical SALATRIM contents calculated from each of the 11 formulations as indicated in Table 2. To incorporate the precision into the accuracy estimation, 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:

$$E = |x - u| + 2.58\text{SD} \quad (9)$$

For each sample, the standard deviation (SD) was calculated from the quadruple analyses of the sample. Table 3 summarizes the precision, accuracy, and total error for each of the 11 samples analyzed. A plot of the calculated SALATRIM contents vs the true values is illustrated in Figure 9. Further statistical analysis indicated that the correlation coefficient (R^2) between the means of 11 calculated values and their corresponding true values was 0.99.

The method used in this study is an application of two established methodologies, a nontraditional crude fat extraction (either SFE or the WCR method) and an IUPAC method of fats and oil analysis. The results showed that the integration of these two methods is well suited for the quantification of generic SALATRIM in foods. The essential analytical procedures of this method involved only the crude fat extraction and the short nonpolar HTCGC analysis as shown in Figure 10. This methodology should be useful for compliance analysis.

Conclusions. SALATRIM developed by the Nabisco Foods Group is a family of low-calorie structured triacylglycerols containing short- and long-chain fatty acids. A generic SALATRIM material contains approximately 95% by weight of triacylglycerols whose acyl carbon numbers (ACN) range from 20 to 42. To develop a method suitable for the determination of generic SALATRIM in foods, 12 food samples (11 samples plus 1 control sample) made with a wide range of SALATRIMs at different concentrations and common vegetable oils, butter fat, and cocoa butter were evaluated. The method used a nontraditional extraction method to obtain a crude fat extract with unaltered triacylglycerols. The crude fat was then subjected to short nonpolar column HTCGC analysis to determine the SALATRIM content of the 11 experimental samples.

The results obtained from this study show that (1) the crude fat contents obtained by the supercritical fluid carbon dioxide extraction and the WCR method were comparable to total fat determined by the AOAC method (a correlation coefficient of 0.98 was achieved); (2) the crude fat obtained was separated into triacylglycerols according to ACNs which were confirmed by the

Table 2. Food Products and Their Corresponding Fat Sources and Contents (Percent) Used in This Study

		SALATRIM 4SO	SALATRIM 24SO	SALATRIM 24SO/4SO	SALATRIM 23SO	soybean oil	butter fat	cocoa butter[e]
	baked product[a]							
1	cookie		4			16		
2	cookie		10			10		
3	cookie		16			4		
4	cookie				4	16		
5	cookie				10	10		
6	cookie				16	4		
7	cookie					20		
	confectionary products							
	nondairy type[b]							
8	chocolate-coated cookie			14.36		10.42		0.65–0.69
9	chocolate-coated cookie				17.24	8.5		0.69–0.83
	dairy type[c]							
10	chocolate-coated butter cookie			18.56			8.88	0.74–0.89
11	chocolate-coated buttercookie				19.17		8.41	0.76–0.92
	frozen dessert[d]							
12	chocolate ice cream	10						0.03–0.05

[a] Baked product: The cookie formulations are shown under Experimental Procedures. [b] Nondairy type confectionery product: chocolate-coated cookie; the formulations are shown under Experimental Procedures. [c] Dairy type confectionery product: chocolate-coated butter cookie; the formulations are shown under Experimental Procedures. [d] Frozen dessert: chocolate ice cream type product; the formulation is shown under Experimental Procedures. [e] Cocoa butter from cocoa powder, which contains 10–12% by weight cocoa butter.

Table 3. SALATRIM Contents in Foods by the Alternative Fat Extraction–HTCGC Analysis

		run 1	run 2	run 3	run 4	av	SD	CV%	theoretical[a] %	accuracy[b] %	total error[c]
	baked products										
1	cookie	4.06	3.84	3.83	3.98	**3.93**	0.11	2.85	**4.00**	−0.1	**0.36**
2	cookie	9.56	9.72	9.73	9.54	**9.64**	0.10	1.05	**10.00**	−0.4	**0.62**
3	cookie	14.54	15.57	15.71	15.06	**15.22**	0.53	3.50	**16.00**	−0.8	**2.15**
4	cookie	4.5	4.41	4.46	4.76	**4.53**	0.16	3.44	**4.00**	0.5	**0.94**
5	cookie	9.38	9.36	9.47	9.45	**9.42**	0.05	0.57	**10.00**	−0.6	**0.72**
6	cookie	16.5	16.69	16.29	16.44	**16.48**	0.17	1.00	**16.00**	0.5	**0.91**
7	cookie[d]	NA	NA	NA	NA	**NA**	NA	NA	**0**	NA	**NA**
	confectionary products										
	nondairy type										
8	chocolate-coated cookie	13.4	13.59	13.58	13.62	**13.55**	0.10	0.74	**14.36**	−0.8	**1.07**
9	chocolate-coated cookie	15.76	18.42	17.72	17.19	**17.27**	1.13	6.53	**17.24**	0.0	**2.94**
	dairy type										
10	chocolate-coated butter cookie	17.2	17.36	16.64	17.26	**17.12**	0.32	1.89	**18.56**	−1.4	**2.28**
11	chocolate-coated butter cookie	18.1	19.74	18.05	18.52	**18.60**	0.79	4.23	**19.17**	−0.6	**2.60**
	frozen dessert										
12	chocolate ice cream	10.63	10.06	10.25	10.03	**10.24**	0.28	2.70	**10.00**	−0.2	**0.95**

[a] Theoretical value (or true value): % SALATRIM by calculation from formulation. [b] Accuracy is expressed as the difference between the calculated average and the theoretical value divided by theoretical value. [c] Total error (E). $E = |x - u| + 2.58\text{SD}$ (*Pharm. Res.* **1991**, *8* (4), 421–426). [d] Not for SALATRIM content analysis.

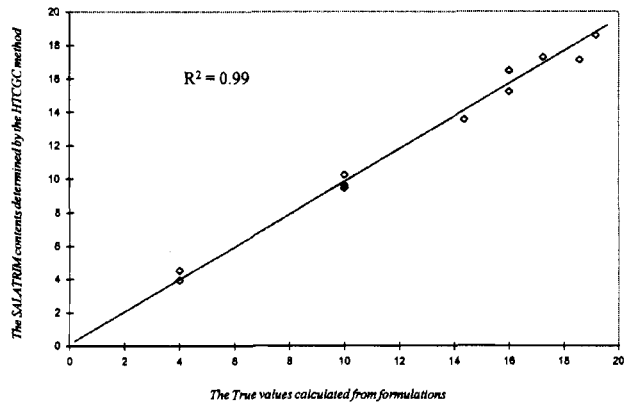

Figure 9. Calculated SALATRIM contents in foods vs the true values from formulations.

HTCGC–mass spectrometry; (3) the generic SALATRIM contents determined by this method were comparable to that calculated from the product formulations witn a correlation coefficient of 0.99.

In food processing research and development, it is often necessary to evaluate food products for their fat ingredients. Ideally, a method determines not only the total fat content but also the origin of the fat. In the current study, we have demonstrated that the generic SALATRIM content of a wide range of food products can be determined with accuracy and precision. This method should be useful as a compliance method.

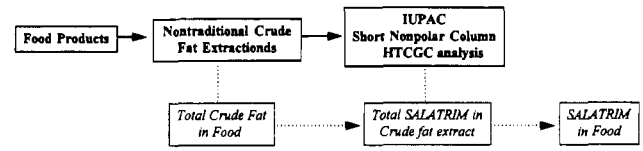

Figure 10. Compliance method for the quantification of generic SALATRIM in foods.

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