Identification and Quantification of SALATRIM 23CA in Foods by the Combination of Supercritical Fluid Extraction, Particle Beam LC–Mass Spectrometry, and HPLC with Light-Scattering Detector

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This paper describes the methodology used in the identification and quantification of SALATRIM 23CA in food. SALATRIM 23CA is a member of the SALATRIM family of triacylglycerols developed by the RJR Nabisco Co. It is an interesterification product of triacetin, tripropionin, and hydrogenated canola oil. Three food items (cookies, candies, and ice cream) made with SALATRIM 23CA and Hydrol 92 coconut oil as their main fat sources were evaluated. The following are reported: (1) How the total fat was extracted from the food items by supercritical fluid carbon dioxide. (2) How the individual triacylglycerols were identified by particle beam LC–mass spectrometry with positive ammonia chemical ionization. (3) How SALATRIM 23CA was separated from other lipid sources and quantified by HPLC with an evaporative light-scattering detector.

INTRODUCTION

The analysis of total fats and oils in food products is a common and extremely important evaluation in the food industry. The evaluation generally involves the quantitative extraction of fats and oils from foods followed by a chemical or physical measurement. An ideal chemical evaluation not only determines the fat content but also determines the fat source and individual triacylglycerols.

The quantitative extraction of fat from lipid-rich food products, such as dairy products, compound coating confectionery products, and cookies, often involves acid/alkaline hydrolysis, multistep extraction procedures, and Soxhlet extraction. Such procedures are time-consuming and often prone to the formation of artifacts. Extraction of fat by supercritical fluid carbon dioxide offers an alternative to traditional extraction methods. The advantages of supercritical fluid extraction (SFE) fat extraction over traditional methods have been discussed in numerous publications (Wright et al., 1988; Friedrich et al., 1982). The fats isolated from food products by SFE are probably the most ideal samples for subsequent fat analysis.

The most popular technique for fat analysis is high-performance liquid chromatography (HPLC). The excellent resolution power of HPLC in lipid separation has been known for some time (El-Hamdy and Perkins, 1981; Smith et al., 1980). It has been enhanced by the introduction of a light-scattering detector for peak detection and quantitation and by the interface with a mass spectrometer for component identification. Peak detection of triacylglycerol is mostly performed by short-wave ultraviolet and refractive index detectors. However, peak detection based on short-wave ultraviolet absorption and refractive index monitoring is restricted to special solvent systems and requires extraordinary care for quantitative work (Kuksis et al., 1991a). The introduction of the light-scattering detector, which is insensitive to solvents and allows direct quantitation, has greatly improved the quality of lipid analysis. Several fat and oil systems have been characterized by HPLC with light-scattering detectors (Palmer and Palmer, 1989).

On-line coupling of HPLC with mass spectrometry requires interfacing the two instruments. This has been accomplished by using any of the following interfaces: direct liquid inlet, moving belt, thermospray, atmospheric pressure ionization, particle beam, and electrospray. In the field of lipid analysis, Kuksis et al. (1991b) identified several triacylglycerol species by reversed-phase liquid chromatography coupled with direct inlet mass spectrometry. Jungalwala et al. (1984) used on-line coupling of HPLC chemical ionization mass spectrometry (CIMS) with a moving-belt interface to identify several molecular species of phospholipid. Kim and Salem (1986) identified several phospholipid species by thermospray liquid chromatography–mass spectrometry. Kusaka et al. (1988) identified a series of fatty acids by LC–MS using the atmospheric pressure ionization technique. With the advances in LC–MS interfacing technology, new interfaces such as the particle beam should provide new vehicles for lipid identification.

In this study, we demonstrate (1) the use of supercritical fluid carbon dioxide to perform a quantitative extraction of fat from foods, (2) the use of an on-line particle beam HPLC–mass spectrometer to identify individual triacylglycerols in SFE fat samples, and (3) the use of HPLC with an evaporative light-scattering detector to quantify SALATRIM 23CA in foods. This combination of three new analytical tools enables scientists to gain a better understanding of the fat systems in foods.

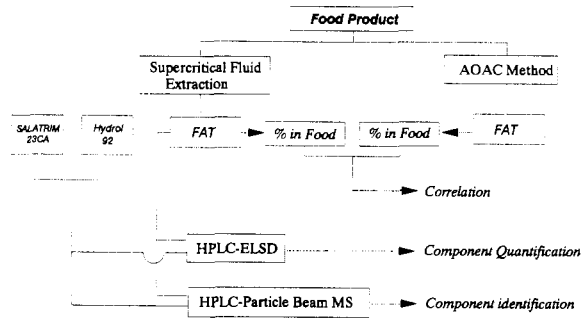

Figure 1. Determination of SALATRIM 23CA in foods.

EXPERIMENTAL PROCEDURES

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

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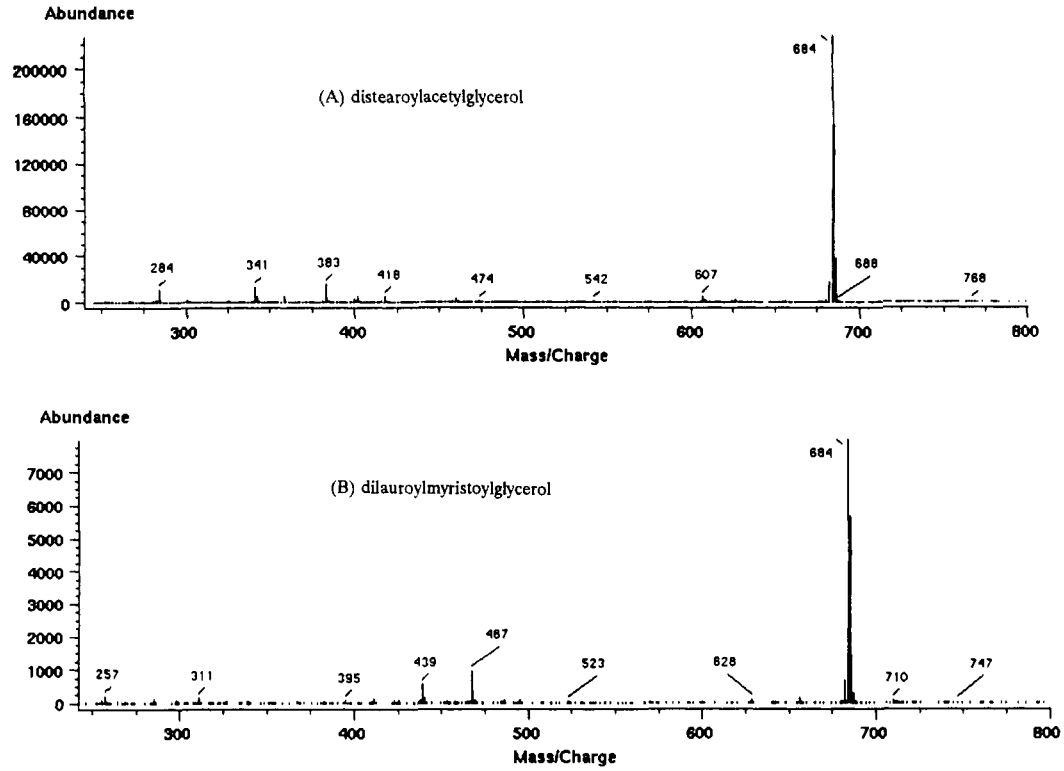

Figure 2. Particle beam LC positive chemical ionization mass spectra of (A) distearoylacetylglycerol and (B) dilauroylmyristoylglycerol. The interpretations are shown in Table 2.

Materials. Three food samples, one sandwich cookie, one compound coating bonbon, and one ice cream, were evaluated. The samples were manufactured with SALATRIM 23CA and Hydrol 92 as their main fat sources. SALATRIM 23CA is a member of the SALATRIM family of triacylglycerols developed by RJR Nabisco. SALATRIM 23CA was obtained from EPL Bio-Analytical Services, Decatur, IL. Hydrol 92 is a refined hydrogenated coconut oil obtained from the Van Den Bergh Foods Co., Lisle, IL.

(*1*) *Sandwich Cookie Sample* (*Sample Code EXSC*). The cream center was made with 50% SALATRIM 23CA, 48.2% sugar, 1.625% whey powder, 0.0687% dough salt, 0.1015% vanillin, and 0.10% cocoa powder. The base cake was obtained from the Consolidated Biscuit Co., McComb, OH. The weight ratios between the base cake and cream center were between 1.05 and 1.22.

(*2*) *Compound Coating Bonbon Sample* (*Sample Code EXCC*). The cream center was the same as in sample EXSC. The compound coating was made with 56.5% sugar, 35% Hydrol 92, 8% cocoa powder, 0.3% lecithin, and 0.2% vanillin. The weight ratios between the cream center and compound coating were 1.8–2.1.

(*3*) *Ice Cream Sample* (*Sample Code EXIC*). The ice cream sample was made with 10% SALATRIM 23CA, 10% nonfat dry milk, 10% sugar, 6% corn syrup (42 DE), 0.3% Dricol, 3% cocoa light, 0.1% lecithin, and 60.6% water.

Fatty Acid Component Determination. The SALATRIM 23CA and Hydrol 92 samples were analyzed for their fatty acid components by gas chromatography of the fatty acid methyl esters (FAME). The procedure was described by Huang et al. (1994).

Fat Content Determined by AOAC Standard Procedures. The fat content of the sandwich cookie sample EXSC and bonbon sample EXCC was determined according to AOAC acid hydrolysis method 14.104 (AOAC, 1984). The fat content of the ice cream sample EXIC was determined by AOAC Roese–Gottlieb method 16.316 (AOAC, 1984). The fat content was expressed in weight percent of the corresponding food item.

SFE Fat Extraction Procedure. Ice cream sample EXIC was first lyophilized with a Labconco Model 8 freeze drier (Labconco Inc., Kansas City, MO) to remove the water. The weight of the ice cream sample before and after freeze-drying was recorded. The sandwich cookie and bonbon samples were used directly for supercritical fluid extraction without dehydration. The cookie, bonbon, and freeze-dried ice cream samples were ground to a fine paste or fine powder. Half a gram (0.5 g) of each sample was placed in a 1.5-mL stainless steel thimble and subjected to 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 being used in the subsequent analysis. The weight of the material collected in the vial was recorded as the fat content by the SFE method. Supercritical carbon dioxide extraction of the food samples was performed with a Hewlett-Packard 7680A extraction module. The SFE conditions were as follows: (*extraction conditions*) extraction fluid, CO_2; density, 0.8 g/mL; pressure, 365 bar; flow, 4 mL/min; temperature, 80 °C; equilibration time, 2 min; extraction time, 30 min; thimble size, 1.5 mL, stainless steel; thimble volumes swept, 92.5; (*trap conditions*) trap material, stainless steel; nozzle temperature, 45 °C; trap temperature, 5 °C; (*fraction output*) rinse solvent, 3 mL (3 × 1 mL) of hexane at 1.0 mL/min; nozzle temperature, 30 °C; trap temperature, 30 °C.

Component Identification by Particle Beam LC–Mass Spectrometry. SALATRIM 23CA, Hydrol 92, and three SFE fat samples were each made into a 6% solution in acetone/THF (50/50 v/v). Acetone and tetrahydrofuran (THF) were purchased from the Sigma Chemical Co., St. Louis, MO. Each sample was analyzed by particle beam LC–mass spectrometry. The analytical conditions were as follows: (*equipment*) Hewlett-Packard 5989A mass spectrometer with a Hewlett-Packard high-energy diode, HP 1090 HPLC system, Hewlett-Packard 59980B particle beam LC–MS interface, and HP59940A ChemStation (HP-UX series, Hewlett-Packard Co., Palo Alto, CA); (*HPLC conditions*) column, Supelcosil LC-18 stainless steel column (15 cm × 2.1 mm i.d.), particle size 5 μm (Supelco Inc., Bellefonte, PA); (solvent) solvent A, acetonitrile, Baker Analyzed HPLC grade solvent; solvent B, *tert*-butyl methyl ether, Baker Analyzed HPLC grade solvent (J. T. Baker Chemical Co., Phillipsburg, NJ); (solvent gradient) 100% solvent A for 10 min (0–10 min); 100% A to 60% A/40% B in 10 min (10–20 min); hold at 60% A/40% B for 10 min (20–30 min); 60% A/40% B to 20% A/80% B in 10 min (30–40 min); (flow rate) constant flow at 0.3 mL/min; (sample injection volume)

2.0 μL; (*interface conditions*) temperature setting at 55 °C, and Nebulizer setting at 7; (*mass spectrometer setting*) ammonia as reagent gas (set at a source pressure of 1.0 Torr) for positive chemical ionization; source temperature, 250 °C; scan range, *m/z* 250–1000 [mass to charge ratios (*m/z*) of the various peaks were determined by the mass marker which had been calibrated with perfluorotributylamine (PFTBA), Hewlett-Packard Co.].

Quantification of SALATRIM 23CA by HPLC with Light-Scattering Detector. SALATRIM 23CA was used to prepare eight standard solutions of different concentrations in THF/acetone (50/50 v/v) with 1% trinonanoin as an internal standard. Trinonanoin (1,2,3-trinonanoylglycerol) was purchsed from Sigma. The eight SALATRIM 23CA concentration levels were 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, and 7% by weight. Each of the three SFE samples was made into a 3% solution in THF/acetone (50/50 v/v) with 1% trinonanoin added. The standard solutions and SFE sample solutions were analyzed by HPLC in triplicate. The conditions used are outlined as follows: (*HPLC system*) Waters 815 HPLC equipped with a Waters 712 WISP autoinjector, a Waters 600E pump (Waters Associates, Milford, MA), and a Varex ELSDII evaporative light-scattering detector (Varex Corp., Rockville, MD); (*column*) two Supelcosil LC-18 stainless steel columns (15 cm × 4.6 mm i.d.) in series; particle size, 5 μm (Supelco); (*solvent*) solvent A, 100% acetonitrile; solvent B, acetonitrile/*tert*-butyl methyl ether (75/25 v/v), *tert*-butyl methyl ether, Baker Analyzed HPLC grade solvent (J. T. Baker) acetonitrile, Baker Analyzed HPLC grade solvent (J. T. Baker); (*solvent gradient*) 100% solvent A (0–20 min), 100% solvent B (20–50 min); (*flow rate*) constant flow at 1 mL/min; (*detector setting*) nebulization in the ELSD was set to 130 °C drift tube temperature (70 °C exhaust temperature) and 70 mm of nitrogen flow to the nebulizer; (*sample size per injection*) 20 μL.

RESULTS AND DISCUSSION

Total Fat Content Determined by SFE and AOAC Standard Methods. The SFE procedure applied in this study was a quantitative extraction analysis. Wright et al. (1988) pointed out that the exhaustive extraction of a sample matrix at a pressure and temperature where all of the components of interest are soluble provides the capability for a quantitative analysis. The triacylglycerol solubility in supercritical fluid carbon dioxide as a function of pressure and temperature has been published by King (1989). The increase in temperature from 40 to 80 °C at 300 atm increases the triacylglycerol solubility in supercritical carbon dioxide (King, 1989). The SFE was set at 80 °C and 365 bar for this study.

To determine the length of time and amount of supercritical CO_2 needed to perform an exhaustive extraction, each sample was subjected to a series of extraction cycles. At the end of each extraction cycle (every 10 min), the sample was weighed and weight loss was recorded. For all three samples tested, no weight loss was observed after 20 min of extraction. An extraction time of 30 min was set for the experiment.

The fat content of each sample as determined by SFE is shown in Table 1. Results showed the precision of the SFE method of ice cream (EXIC) fat determination to be 3.9% coefficient of variation (CV). The CVs of the rest of the samples were below 3%. A comparison of fat content as determined by the AOAC and SFE methods showed that the SFE results were comparable to those obtained by AOAC methods.

The primary method presently used to determine the fat content in foods is the AOAC extraction method. The AOAC fat determination of baked goods, such as cookies, involves acid hydrolysis and high-temperature solvent removal steps. Fat determination of dairy products involves alkaline treatments and solvent removal procedures. Such procedures are prone to the formation of artifacts. Supercritical extraction of fat, on the other hand, does not require the acid/alkaline hydrolysis step. As a result, these fats are more suitable for follow-up evaluation.

Table 1. Total Fat Contents Determined by the Supercritical Carbon Dioxide Extraction and by the AOAC Methods[a]

	supercritical fluid CO_2 extraction						
sample	run 1	run 2	run 3	av[b]	s[c]	CV[d]	AOAC av[e]
EXSC[f]	33.8	34.5	34.6	34.3	0.4	1.3	34.6
EXCC[g]	46.2	46.1	48.3	46.8	1.2	2.7	47.4
EXIC[h]	11.9	11.4	11.0	11.4	0.5	3.9	11.1

[a] Fat content was expressed by the weight percent of fat in corresponding sample. [b] Average of three measurements. [c] Standard deviation. [d] Coefficient of variation. [e] Average of three measurements by the AOAC method. [f] Experimental sandwich (description under Experimental Procedures). [g] Experimental compound coating candy (description under Experimental Procedures). [h] Experimental ice cream (description under Experimental Procedures).

Identification of Major Triacylglycerol Species in SALATRIM 23CA, Hydrol 92 Coconut Oil, and SFE Extracted Fat Samples. *Fatty Acid Determination.* SALATRIM 23CA is an interesterification product of triacetin (1,2,3-triacetylglycerol), tripropionin (1,2,3-tripropanylglycerol), and hydrogenated canola oil at a molar ratio of 11:1:1. It has the following fatty acids: acetic (C2:0) acid, propionic (C3:0) acid, palmitic (C16:0) acid, stearic (C18:0) acid, oleic (C18:1) acid, arachidic (C20:0) acid, behenic (C22:0) acid, and lignerceric (C24:0) acid (Huang et al., 1993). Hydrol 92 hydrogenated coconut oil has the following fatty acids: caproic (C6:0) acid, caprylic (C8:0) acid, capric (C10:0) acid, lauric (C12:0) acid, myristic (C14:0) acid, palmitic (C16:0) acid, stearic (C18:0) acid, oleic (C18:0) acid, linoleic (C18:2) acid, arachidic (C20:0) acid, and behenic (C22:0) acid. The fatty acid data of the above materials were used to assist in the identification of triacylglycerols in the particle beam LC–MS analysis.

Particle Beam LC–MS Analysis. The positive ammonia chemical ionization spectra were used to determine the molecular structures of major components in SALATRIM 23CA, Hydrol 92 coconut oil, and SFE fat samples. The objective was to positively correlate each of the SFE fat components to their corresponding fat sources.

In the positive $[NH_3]$CIMS analysis of triglycerides, triacylglycerol with molecular weight M produces the pseudomolecular ion $[M + NH_4]^+$, fragment ions $[MH - RCOOH]^+$, and acylium ion $[RCO + 74]^+$. Fragment ion $[MH - RCOOH]^+$ represents the loss of one fatty acid from protonated molecular ion $[M + H]^+$. 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. Such mechanisms can be demonstrated by two mass spectra obtained from this study. Distearoylacetylglycerol and dilauroylmyristoylglycerol both have pseudomolecular ions $[M + NH_4]^+$ at *m/z* 684 (Figure 2). Both compounds were calculated to have a molecular weight of 666. Distearoylacetylglycerol has fragment ions at *m/z* 607 and 383 resulting from the loss of acetic acid and stearic acid from the protonated molecular ion $[M + H]^+$, respectively. The ion at *m/z* 341 corresponds to the acylium ion $[RCO + 74]^+$ of the C18:0 fatty acid. On the other hand, dilauroylmyristoylglycerol has fragment ions at *m/z* 467 and 439 resulting from the loss of lauric acid and myristic acid from the protonated molecular ion $[MH]^+$, respectively. The ions at *m/z* 257 and 285 correspond to the acylium ion lauric acid and myristic

Table 2. Triacylglycerol (TG) Identification by PB LC–MS[a]

identification		characteristic LC–MS mass/charge, m/z				
ID[b]	TG species	$[M+18]^+$	MW[c]	$[MH-RCOOH]^+$	$[RCO+74]^+$	fatty acid
S-1	Ac–S–OH[d]	418	400			
	Ac–Ac–P[e]	432	414			
	Ac–Pr–P[f]	446	428			
S-2	Ac–S–Ac[g]	460	442	383		C2:0
					341	C18:0
S-3	Ac–Pr–S[h]	474	456	397		C2:0
				383		C3:0
					341	C18:0
S-4	Ac–P–S[i]	656	638	579		C2:0
				383	313	C16:0
				355	341	C18:0
S-5	Ac–S–S[j]	684	666	607		C2:0
				383	341	C18:0
C-1	Cy–La–La[k]	600	582	439	201	C8:0
				383	257	C12:0
C-2	C–La–La[l]	628	610	439	229	C10:0
				411	257	C12:0
C-3	La–La–La[m]	656	638	439	257	C12:0
C-4	La–La–M[n]	684	666	467	257	C12:0
				439	285	C14:0
C-5	La–M–M[o]	712	694	495	257	C12:0
				467	285	C14:0
C-6	M–M–M[p]	740	722	495	285	C14:0

[a] Identified by particle beam LC–mass spectrometry (from samples SALATRIM 23C, Hydrol 92, and SFE). [b] Compounds S-1–S-5 are components of SALATRIM 23CA, and compounds C-1–C-6 are components of Hydrol 92. Peak identifications are shown in Figure 3. [c] Molecular weight calculated from $[M+NH_4]^+$. [d] Acetylstearoylglycerol. [e] Diacetylpalmitoylglycerol. [f] Acetylpropionylpalmitoylglycerol. [d–f] Components were not well separated in HPLC analysis. [g] Diacetylstearoylglycerol. [h] Acetylpropionylstearoylglycerol. [i] Acetylpalmitoylstearoylglycerol. [j] Distearoylacetylglycerol (consists of two positional isomers, 1,3-distearoyl-2-acetylglycerol and 1,2-distearoyl-3-acetylglycerol). [k] Dilauroylcaprylolylglycerol. [l] Dilauroylcaproylglycerol. [m] Trilaurin. [n] Dilauroylmyristoylglycerol. [o] Dimyristoyllauroylglycerol. [p] Trimyristin. [d–p] Fatty acid abbreviations: Ac, acetic acid; Pr, propionic acid; Cy, caprylic acid; La, lauric acid; C, capric acid; M, myristic acid; P, palmitic acid; S, stearic acid.

Table 3. Calculated SALATRIM 23CA Contents and Original Added Values in Foods

	calcd SALATRIM 23CA contents, wt %						
sample	run 1	run 2	run 3	av[d]	s[e]	exptl value[f]	wt % theor value[g]
EXSC[a]	21.7	23.2	22.8	22.6	0.7	22.6 ± 0.7	21.2–23.1[h]
EXCC[b]	33.3	34.6	30.5	32.8	2.1	32.8 ± 2.1	30.1–35.0[i]
EXIC[c]	10.1	10.8	10.7	10.5	0.4	10.5 ± 0.4	10.0[j]

[a] Experimental sandwich cookies. [b] Experimental compound coating candy. [c] Experimental ice cream. [d] Average of three measurements. [e] Standard deviation; the coefficients of variation were 3.2%, 6.4%, and 3.4% for samples EXSC, EXCC, and EXIC, respectively. [f] Experimental results. [g] Values calculated from the original formulations. [h] Based on the ratios of cream center and base cake varied from 42.4:57.6 to 46.2:53.8. [i] Based on the cream center and compound coating varied from 60.2:40.8 to 70:30. [j] Based on the ice cream formulation which contained 10% SALATRIM 23CA; the sample variation data were unavailable.

acid, respectively. Distearoylacetylglycerol is a component of SALATRIM 23CA, and dilauroylmyristoylglycerol is a component of Hydrol 92. The two triacylglycerols, with identical molecular weights, were well separated by HPLC (Figure 3D).

Five SALATRIM 23CA major components and six Hydrol 92 major components were identified by PCI mass spectrometry. The identified compounds with their characteristic ions and fatty acids are summarized in Table 2. Each identified component was positively correlated to its fat source. The detailed identification of triacylglycerols in SALATRIM 23CA was reported in another study (Huang et al., 1994).

HPLC Analysis of SFE Fat Samples. Five HPLC profiles representing SALATRIM 23CA, Hydrol 92 coconut oil, and three SFE samples are shown in Figures 3. Each individual component was related to its corresponding fat source, SALATRIM 23C, Hydrol 92, and others. As expected, the sandwich cookie (EXSC) and ice cream (EXIC) samples contained only SALATRIM 23CA components and none of the Hydrol 92 coconut oil components. On the other hand, the bonbon sample (EXCC) contained both SALATRIM 23CA and Hydrol 92 coconut oil components. In the bonbon sample, the outerlayer coating was made with Hydrol 92 coconut oil and the cream center was made with SALATRIM 23CA.

SALATRIM 23CA Quantitation. The SALATRIM 23CA calibration curve was established from the peak area ratios (Y) of total SALATRIM 23CA peak areas to the internal standard trinonanoin peak area and from the weight ratios (X) of SALATRIM 23CA to trinonanoin. The total peak area of SALATRIM 23CA is the sum of peak areas from all SALATRIM 23CA individual components. Similar quantitation approaches utilizing HPLC with light-scattering detector in lipid analysis have been demonstrated by several authors (Christie, 1985; Stolyhwo et al., 1985; Letter, 1992). Some publications used linear regression (concentration vs response) models to quantify the lipids, and a fairly good approximation of quantitation was obtained. However, Stolyhwo et al. (1985) showed that the response of the light-scattering detector is not entirely linear but proportional to some power of the concentration of the corresponding compound in the analyzed sample. In this study, the calibration curves based on the linear model and nonlinear model were compared. The exponential curve (Figure 4) has a better curve fit than that of the linear curve ($r^2 = 0.996$). The nonlinear regression equation was

$$Y = -0.28 + 0.55 \times X^{1.31} \quad (r^2 = 0.999)$$

The ratio (X) of SALATRIM 23CA total peak area to internal standard peak area in each of the SFE fat samples was used to calculate the SALATRIM 23CA content in food. The SALATRIM 23CA content was calculated according to the calibration curve.

The weight percentages of SALATRIM 23CA in each

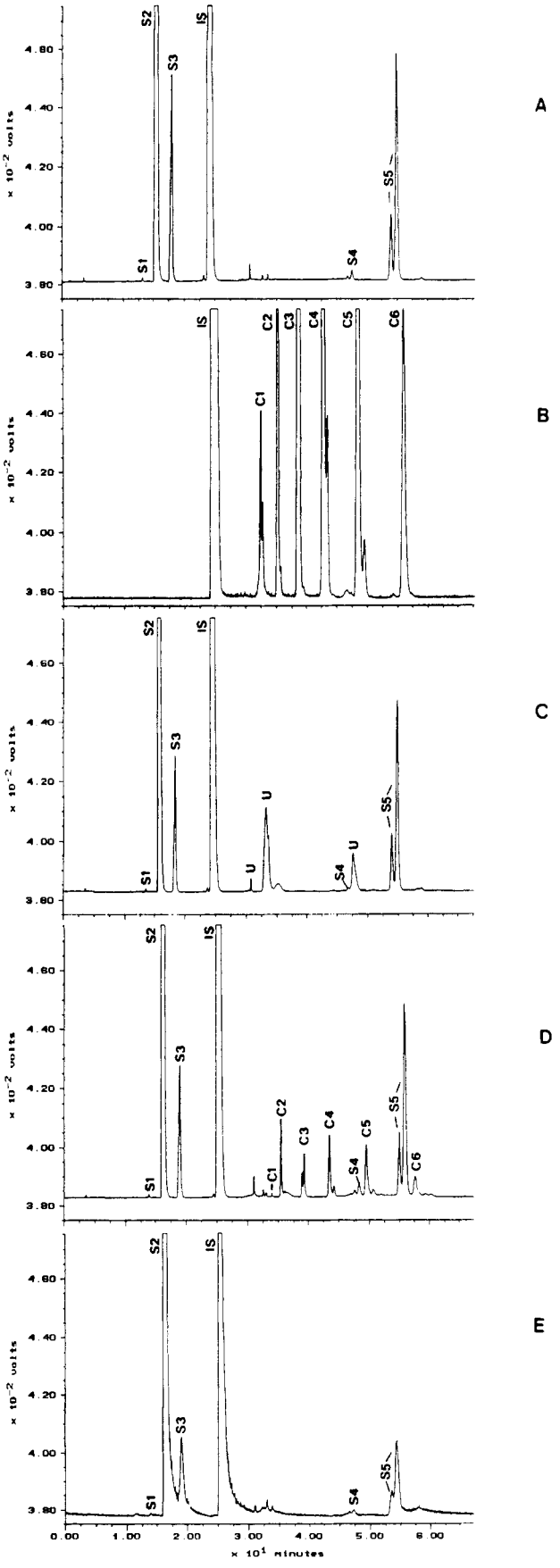

Figure 3. HPLC profiles of (A) SALATRIM 23CA, (B) Hydrol 92, (C) SFE extract of sample EXSC, (D) SFE extract of sample EXCC, and (E) SFE extract of sample EXIC. Identifications of peaks S1, S2, S3, S4, S5, C1, C2, C3, C4, C5, and C6 are given in Table 2. Internal standard (IS) was trinonanoin. (U) Unknown peaks.

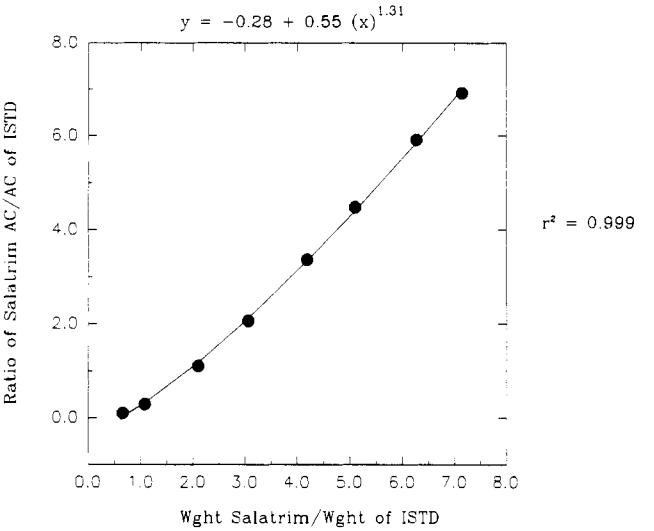

Figure 4. Calibration curve of weight ration (X) of SALATRIM 23CA to trinonanoin and peak area ratio (Y) of SALATRIM 23CA to trinonanoin. Trinonanoin is the internal standard.

of the three food samples are listed in Table 3. The coefficient of variation of each sample, except sample EXCC (CV = 6.4%), was below 5%. The high CV for the bonbon sample is probably due to a lack of consistency in the layering of the compound coating on the product. The SALATRIM 23CA results obtained by this approach are all within the ranges of theoretical values (Table 3).

Conclusions. The results obtained from this study show that a single quantitative supercritical fluid carbon dioxide extraction accomplished two objectives: (1) the determination of the total fat content in foods and (2) the preparation of fat samples that were suitable for subsequent analysis. The fat contents determined by the SFE method were comparable to those determined by the standard AOAC method. For subsequent fat evaluation, reversed-phase high-performance liquid chromatography provided enough resolution to separate SFE samples into individual triacylglycerols. On-line particle beam LC–mass spectrometry revealed the identities of each individual triacylglycerol. This enabled the individual triacylglycerols to be related to their corresponding fat sources. HPLC with an evaporating light-scattering detector was the tool used for the quantification of SALATRIM 23CA. The weight percentages of SALATRIM 23CA obtained by this analytical approach were within the ranges of the original added values.

In food-processing research and development, it is often necessary to evaluate the fat ingredients after the manufacturing process. This study proved that the combination of several analytical tools can accomplish this objective.

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LITERATURE CITED

AOAC. Fat in Bread—Acid Hydrolysis Method—Final Action. In *Official Methods of the Association of Official Analytical Chemists*; AOAC: Arlington, VA, 1984; Method 14.104.

AOAC. Fat in Ice Cream and Frozen Desserts—Roese-Gottlieb Method—Final Action. In *Official Methods of the Association*

of Official Analytical Chemists; AOAC: Arlington, VA, 1984; Method 16.316.

Christie, W. W. Rapid Separation and Quantitation of Lipid Classes by High Performance Liquid Chromatography and Mass (Light-Scattering) Detection. *J. Lipid Res.* **1985**, *26*, 507–512.

El-Hamdy, A. H.; Perkins, E. G. High Performance Reversed-Phase Chromatography of Natural Triglyceride Mixtures. *J. Am. Oil Chem. Soc.* **1981**, *58*, 49–53.

Friedrich, J. P.; List, G. R.; Heakin, A. J. Petroleum-free Extraction of oil from Soybeans with Supercritical CO_2. *J. Am. Oil Chem. Soc.* **1982**, *59*, 288–292.

Huang, A. S.; Delano, G. D.; Pidel, A.; Janes, L.; Softly, B. J.; Templeman, G. Characterization of Triacylglycerols in Saturated Lipid Mixtures with Application to SALATRIM 23CA. *J. Agric. Food Chem.* **1994**, one of several papers in this issue.

Itabashi, Y.; Marai, L.; Kuksis, A. Identification of Molecular Species of Enantiomeric Diacylglycerols by Chiral Phase LC/CIMS of the 3,5-Dinitrophenylurethans. *J. Am. Oil Chem. Soc.* **1989**, *66*, 491.

Jungalwala, F. B.; Evans, J. E.; McCluer, R. H. Compositional and Molecular Species Analysis of Phospholipid by High Performance Liquid Chromatography Coupled with Chemical Ionization Mass Spectrometry. *J. Lipid Res.* **1984**, *25*, 738–749.

Kim, H. Y.; Salem, N., Jr. Phospholipid Molecular Species Analysis by Thermospray Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* **1986**, *58*, 9–14.

King, J. W. Fundamentals and Applications of Supercritical Fluid Extraction in Chromatographic Science. *J. Chromatogr. Sci.* **1989**, *27*, 355–364.

Kuksis, A.; Marai, L.; Myher, J. J.; Itabashi, Y.; Pind, S. Qualitative and Quantitative Analysis of Molecular Species of Glycerolipids by HPLC. In *Analyses of Fats, Oils and Lipoproteins*; Perkins, E. G., Ed.; American Oil Chemists' Society: Champaign, IL, 1991a; Chapter 13.

Kuksis, A.; Marai, L.; Myher, J. J. Reverse-Phase Liquid Chromatography-Mass Spectrometry of Complex Mixtures of Natural Triacylglycerols with Chloride-Attachment negative Chemical Ionization. *J. Chromatogr.* **1991b**, *588*, 73–87.

Kusaka, T.; Ikeda, M.; Nakano, H.; Numajiri, Y. Liquid Chromatography/Mass Spectrometry of Fatty Acids as Their Anilides. *J. Biochem.* **1988**, *104*, 495–497.

Lauer, W. M.; Aasen, A. J.; Graff, G.; Holman, R. T. Mass Spectrometry of Triglycerides: I. Structural Effects. *Lipids* **1970**, *5*, 861–868.

Letter, W. S. A Rapid Method for Phospholipid class Separation By HPLC Using An Evaporative Light-Scattering Detector. *J. Liq. Chromatogr.* **1992**, *15*, 253–266.

Palmer, A. J.; Palmer, F. J. Rapid Analysis of Triacylglycerols using High-Performance Liquid Chromatography with Light Scattering Detection. *J. Chromatogr.* **1989**, *465*, 369–377.

Smith, E. C.; Jones, A. D.; Hammond, E. W. Investigation of The Use of Argentation High-Performance Liquid Chromatography for The Analysis of Triglycerides. *J. Chromatogr.* **1980**, *188*, 205–212.

Stolyhwo, A.; Colin, H.; Guiochon, G. Analysis of Triglycerides in Oils and Fats by Liquid Chromatography with the Laser light Scattering Detector. *Anal. Chem.* **1985**, *57*, 1342–1354.

Wright, B. W.; Fulton, J. L.; Kopriva, A. J.; Smith, R. D. Analytical Supercritical Fluid Extraction Methodologies. In *Supercritical Fluid Extraction and Chromatography, Techniques and Applications*; Charpentier, B. A., Sevenants, M. R., Eds.; ACS Symposium Series 366; American Chemical Society: Washington, DC, 1988; Chapter 3.

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