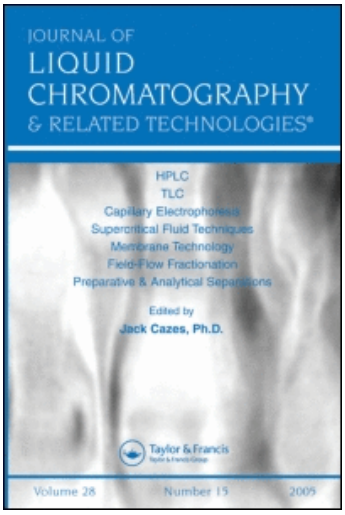

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TRIACYLGLYCEROL STRUCTURES OF FOOD FATS HIGH IN SATURATED ACIDS BY HPLC AND MASS SPECTROMETRY

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ABSTRACT

Triacylglycerol (TAG) compositions by area percent were obtained by reverse-phase high-performance liquid chromatography (RP-HPLC) coupled with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) of highly saturated fatty acid fats, such as coconut, cocoa butter, palm, randomized palm, palm olein, and randomized palm olein oils. Accurate identification and quantitation of these TAG compositions were obtained and proved by comparison of the fatty acid composition calculated from the TAG composition obtained by APCI-MS with the fatty acid composition obtained by gas chromatography of the methyl esters of the transmethylated oils. Also, APCI-MS accuracy was proved by comparison of the experimental TAG composition with the predicted TAG composition for randomized oils. Average absolute errors, with respect to TAG quantitation and identification, were less than 1%. Our study identified and quantitated these TAGs present at greater than 0.1% (oil, number of TAGs):

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coconut, 99; palm, 27; randomized palm, 28; palm olein, 28; randomized palm olein, 29, and cocoa butter, 19. Concentrations of UUU, UUS, USS, and SSS TAGs, which can be determined accurately from RP-HPLC/APCI-MS of the actual TAG species, affected the physical properties of food formulation fats.

INTRODUCTION

Improvement of the functional properties of vegetable fats through the development of new fats with increased amounts of saturated fatty acids, such as high palmitic and stearic acid soybean and high stearic and lauric acid canola oils (1–5), or the chemical- or lipase-catalyzed modification of traditional commodity oils (6–9) for food formulation products, such as margarine base stocks and confectionery products, has been in progress. However, traditional fats such as coconut, cocoa butter, palm, randomized palm, palm olein, and randomized palm olein oils remain important (10).

The triacylglycerol (TAG) composition (i.e., kinds and quantities of individual TAGs) and TAG structure [i.e., kinds and quantities of individual fatty acids (FAs) located at the TAG glycerol moiety carbons] affect the food formulation product functional properties, such as melting point range, solid fat index, and crystal structure. These physical properties affect food properties from texture to taste (10,11). Also, the fat oxidative stability is, in part, dependent on TAG composition and structure (12–15).

The oxidative stability affects the storage as well as the nutritional and safety stability of the food product. Also, altered fats are investigated as substitutes for presently important commercial fats, such as coconut and cocoa butter in confectionery and other food formulation products (16). Thus, it is important to know the TAG composition of the altered fats, compared to the TAG composition of coconut and cocoa butter. In summary, the knowledge of the kinds and quantities of individual TAGs in vegetable oils is important in food chemistry.

Previously, for qualitative TAG analysis, identification of TAGs resolved by reverse-phase high-performance liquid chromatography (RP-HPLC) of fats, was conducted by collection of HPLC fractions for subsequent gas chromatography identification of the TAG methyl esters after transmethylation or by matching HPLC retention times or volumes with TAG equivalent carbon numbers with respect to standard TAGs (17–19). Recently, RP-HPLC, coupled with atmospheric pressure chemical ionization mass spectrometry (APCI-MS), has been used to conclusively identify eluting TAGs during RP-HPLC of fats (20,21).

MS of the TAGs through use of the APCI-MS method gave spectra which contained a simplified number of very distinctive fragment ions including diacylglycerol, protonated molecular ions, and molecular related ions that conclusively

identified individual TAGs in vegetable oil TAG mixtures. Thus, this new methodology permitted the facile determination of the kinds of TAGs in many vegetable oil mixtures (VGO).

For quantitative TAG analysis, the weakness has been the method of detection, since TAGs do not have strongly chromatophoric groups and the gradient solvent system needed for RP-HPLC TAG resolution presents absorbance problems for the commonly used HPLC ultraviolet absorbance detector. Christie and others (22–27) have written extensive reviews on possible HPLC detectors for quantitative TAG analysis. The consensus of these authors is, that while not perfect, the HPLC flame ionization detector (FID) and evaporative light scattering detector (ELSD) are the preferred detectors for quantitative TAG analysis.

We have made extensive use of a FID for quantitative HPLC analysis of individual TAGs in VGO mixtures (17,19). This detector allowed TAG quantitation without the need for detector response factors. Thus, TAG quantitation was obtained as area percent, which for the FID is related to weight percent, obtained by computer integration of the RP-HPLC TAG chromatogram peak areas. Accuracy of the TAG composition could then be checked by comparison of the FA composition calculated from the experimental TAG composition against experimental FA composition obtained from gas chromatography (GC) analysis of the transmethylated VGO mixture.

We have also used APCI-MS as a quantitative detector for RP-HPLC of VGO (20,21). This detector was determined to give quantitative results for TAGs through facile calculation of individual response factors of individual TAGs based on the raw MS response and the FA composition previously obtained by GC of the VGO mixture.

Also, since a mass spectrometer is being used as the HPLC detector, selective ion monitoring can be used for identification and quantitation of unresolved TAGs. Thus, RP-HPLC coupled with a quadrapole mass spectrometer through an atmospheric pressure chemical ionization source can more completely identify and quantitate all TAGs present in a vegetable oil than other TAG identification techniques.

It is important to know accurately the TAG structure of coconut, cocoa butter, palm, randomized palm, palm olein, and randomized palm olein oils in the development of food formulation products for confectionery, shortening, margarine, and other food products. In this report, the TAG compositions of these oils were obtained by RP-HPLC coupled with APCI-MS as a HPLC detector. Accurate identification and quantitation of these TAG compositions were obtained and proved by comparison of the fatty acid composition calculated from the TAG composition obtained by APCI-MS with the fatty acid composition obtained by GC of the methyl esters of the transmethylated oils. Also, APCI-MS accuracy was proved by comparison of the experimental TAG composition with the predicted TAG composition for randomized oils.

EXPERIMENTAL

Materials

The coconut, cocoa butter, palm olein, and palm oil samples were obtained from either local market or industrial sources as refined, bleached, deodorized finished oils. Randomized oils were prepared in the presence of sodium methoxide as a catalyst (28).

HPLC mobile solvents, acetonitrile (ACN) and dichloromethane (DCM), were HPLC grade and were purchased from EM Science (Gibbstown, NJ, USA) and Fisher Scientific (Fairlawn, NJ, USA), respectively. They were used without further purification.

High-Performance Liquid Chromatography

The HPLC system used for RP-HPLC/APCI-MS, contained a LDC 4100 mass spectrometer (Thermo Separation Products), and a quaternary pump with membrane degasser, which was equipped with two in-series RP-HPLC columns [25 × 0.46 cm, with bonded silyl (CT8) ODS, 5-μm particle size, Inertsil ODS-80A; GL Sciences, Keystone Scientific-Bellefonte Park, PA, USA).

The gradient used for separation of the TAG components was as follows: initial conditions 70% ACN/30% DCM; from 0 to 20 min, then linear from 20 to 40 min to 60% ACN/40% DCM, kept at 60% ACN/40% DCM to 50 min, than linear to 40% ACN/60% DCM at 70 min, kept at 40% ACN/60% DCM to 75 min, than linear to 30% ACN/70% DCM at 80 min, kept at 30% ACN/70% DCM to 85 min, then linear return to initial conditions, 70% ACN/30 DCM at 99 min. The flow rate was 0.7 mL/min throughout. Flow was split, using a tee, so that ~680 μL/min went to an ELSD and ~120 μL/min went to the mass spectrometer.

A Varex MKIII ELSD detector (Alltech Associates, Deerfield, IL, USA) was used as an auxiliary detector for RP-HPLC/APCI-MS. The drift tube was set to 140°C. The gas flow was 2.0 standard L min. High purity N_2 was used as the nebulizer gas.

ELSD output was simultaneously directed to a stand-alone data system with 24-bit resolution (EZ-Chrome Elite; Scientific Software, Inc., Pleasanton, CA, USA). Injections of 10 mL were made using a series 1050 autosampler (Hewlett-Packard, Wilmington, DE, USA).

Atmospheric Pressure Chemical Ionization Mass Spectrometer Detector

Identification and quantitation of TAG components were performed with APCI-MS. A MAT TSQ700 (Finnigan, San Jose, CA, USA) mass spectrometer

operating in Q1 low-mass mode was used for acquisition of APCI-MS data. The APCI-MS vaporizer was operated at 400°C, the capillary heater was operated at 265°C, and the corona voltage was set to 6.0 mA. Sheath and auxiliary gases were set to 35 psi and 5 mL/min, respectively. Spectra were obtained from 400 to 1100 amu with a scan time of 1.75–2.0 s.

Chromatograms were processed using three-point smoothing for graphical output, but no smoothing was applied during quantitation of extracted ion chromatograms. All mass spectra shown represent an average of spectra over the breath of a chromatographic peak. Nominal masses, shown in mass spectra, were obtained by application of a mass defect of 0 mmu at 0 amu to 700 mmu at 1000 amu.

Gas Chromatography

Fatty acid methyl esters (FAMEs) were prepared by 0.5 *N* hydrochloric acid in methanol transmethylation of the TAG mixtures (29). For oils with shorter chain fatty acids (C:6, 8, and 10), such as coconut oil, to avoid loss of more volatile acids, the benzene/methanol was only water-washed to remove the hydrochloric acid. Without further work, up to a 5-μL portion of the benzene layer was injected directly onto the gas chromatograph. The FAMEs were analyzed using calibrated GC according to this procedure. A 0.5-μL FAME sample solution (5 mg of sample per mL of hexane) was analyzed by direct injection capillary GC. The capillary column was a SP2380 column, 30 m ×·0.25 mm inside-diameter with 0.2-μm film thickness (Supelco, Inc., Bellefonte, PA, USA).

The gas chromatograph was a Star model 3400, equipped with a flame ionization detector (Varian, Inc., Walnut Creek, CA, USA). The GC column was operated at a starting temperature of 150°C, except for coconut oil, which required a starting temperature of 75°C. The column was programmed at 150°C, held for 35 min, then heated at 2°C/min to 210°C, and then to 220°C and held at 220°C for 5 min. The helium carrier gas had a column head pressure of 15 psi. The injector and detector were maintained at 240 and 280°C, respectively. GC calibration mixtures were FAME mixture 20A for all oils, except coconut oil, which required FAME mixture 4°C (C:6, 8, 10, 12, and 14 FAMEs) (NU-CHEK PREP, Inc., Elysian, MN, USA).

The GC analyses were used to calibrate the crude mass spectrometric data to provide accurate quantitation of TAG components.

Physical Property Tests

The solid fat index (SFI) and melting points were determined by the American Oil Chemists' Society official methods (30).

RESULTS AND DISCUSSION

TAG compositions by area percent were obtained by RP-HPLC/APCI-MS of highly saturated fatty acid fats, such as coconut, cocoa butter, palm, randomized palm, palm olein, and randomized palm olein oils. These fats, often in combination with corn, cottonseed, and soybean oils or their derivatives, such as cottonseed oil hard stock, are used in products for confectionery, shortening, margarine base stocks, and other food products. Accurate identification and quantitation of these saturated fatty acid TAG compositions are needed to understand the TAG effect on food lipid physical properties, such as solid fat content and melting point (31).

Identification of individual TAGs was possible because MS with the APCI source gave easy-to-interpret mass spectra with primarily TAG-identifying diglyceride fragments and molecular ion information. The simple appearance of spectra and the use of extracted ion chromatograms of characteristic diglyceride fragments and molecular ions made qualitative and quantitative analysis straight +forward and facile.

The accuracy of TAG identification and quantitation was proved by comparison of the fatty acid composition calculated from the response factor-corrected TAG composition, obtained from APCI-MS of the oils, with the fatty acid composition obtained by GC of the methyl esters of the transmethylated oils. Also, APCI-MS accuracy was proved by comparison of the experimental TAG composition with the predicted TAG composition for randomized oils.

TAG identification from the APCI-MS spectra was based on mass spectra, which showed that minimal fragmentation occurred (32). The fragmentation resulted primarily in diglyceride $[M\text{-}RCOO]^+$ ions and [M + 1] protonated ions. The degree of fatty acid unsaturated in the TAGs had a marked effect on the proportion of diglyceride compared to protonated molecular ions. Mass spectra of triglycerides, which contained fatty acids with two or three double bonds, showed protonated molecular ions as the abundant ions with diglyceride peaks representing 13–25% of the base peak. The triglycerides, which contained fatty acids with one double bond, produced diglycerides as the base peak and [M + 1] ions with intensity of 20–28% of the base peak. No [M + 1] ions were found in the spectra of triglycerides that contained only saturated fatty acids, and only the characteristic TAGs identifying diglyceride pairs. Extracted ion chromatograms were used to identify those TAGs that had the same RP-HPLC retention.

For TAG quantitation via APCI-MS data, the following procedure was used. The total ionization chromatogram area count for each TAG was obtained by the sum of the areas under all peaks of fragments arising from a particular TAG plus the area under the mass of the protonated molecular ion. This gave raw or uncorrected TAG composition per oil. The amount of fragmentation in APCI-MS spectra has been shown to strongly depend on the degree of unsaturation in

the TAG. Because of this, quantitation of TAGs by APCI-MS also has been shown to depend on the degree of unsaturation in the TAG. TAGs that contain a high degree of unsaturation produced more molecular ion and give less overall response, while those TAGs that are more saturated give mostly $[M\text{-}RCOO]^+$ fragments and larger chromatogram peak areas to represent these fragments. The saturates tend to be over-represented in percent compositions, while the unsaturates tend to be under-represented. To solve this, a method for calculation of response factors for TAGs determined by APCI-MS was developed (33).

Response factors were calculated for each fatty acid by dividing the FA composition obtained by calibrated GC-FID of the transmethylated oil by the FA composition calculated from the uncorrected TAG composition. The response factors were thus, calculated for an oil TAG by calculating the ratio of the FA composition obtained by GC-FID to the FA composition calculated from the raw TAG composition obtained by APCI-MS.

These data were then normalized to one of the FAs set equal to 1.0, which was usually the FA with the least area percent (unless it was present at a very low level, in which case the FA with the smallest area percent over 1% was used). Using these FA response factors, TAG response factors were calculated by multiplying the FA response factors together. For example, in the case of coconut oil, the TAG response factor for LaLaLa (trilaurin) was 1.0000, while that for LaLaO was 1.1361. When the full set of TAG response factors was applied to the raw APCI-MS data, the adjusted or corrected TAG area percent, given in Table 1, resulted for coconut oil. This corrected TAG composition was possible because FA response factors were multiplied together to give TAG response factors, which when applied to the uncorrected TAG composition gives a TAG composition, which has been demonstrated to have the lowest average relative error compared to other methods (20,33). This method for TAG quantitative analysis is extended to the present study of the triglyceride compositions of food formulation products.

Accuracy of TAG identification and quantitation for each oil is indicated by agreement of the fatty acid composition calculated from the corrected TAG composition with the experimental fatty acid composition obtained by GC of the methyl esters obtained from the transmethylated oil.

Coconut Oil

This oil contained nine low to high boiling point FAs, which were 0.7% capric (Co), C6:0; 8.1% caprylic (Cy), C8:0; 6.3% caproic (Ca), C10:0; 48.3% lauric (La), C10:0; 18.1% myristic (M), C14:0; 8.4% palmitic (P), C16:0; 1.8% linoleic (L), C18:2; 5.9% oleic (O), C18:1; and 2.4% stearic (S), C18:0 acids, as determined by calibrated GC of the FAMEs from the transmethylated oil.

Table 1. Coconut Oil TAGs Determined by RP-HPLC Coupled with APCI-MS[a]

TAG[b]	Abs. Ret. Time[c]	% Comp.[d]	TAG	Abs. Ret. Time	% Comp.	TAG	Abs. Ret. Time	% Comp.	TAG	Abs. Ret. Time	% Comp.
LaLaM	22:11	12.8	OOP	42:51	0.7	CaLaP	17:05	0.3	CaOL	20:51	0.1
LaLaLa	19:09	9.2	CaCaLa	14:36	0.6	LaPL	27:36	0.3	SOL	41:52	0.1
LaLAC	14:36	8.3	CaMP	26:01	0.6	CyMS	25:59	0.3	CoMP	19:39	0.1
CyLaM	16:46	7.5	MPO	39:24	0.6	LaLaL	20:28	0.3	CyCyP	14:41	0.1
CaLaM	19:15	5.6	CaLaS	25:59	0.6	CyPO	24:32	0.2	CaCaP	19:20	0.1
LaLaP	26:01	4.6	LaLaCo	13:09	0.5	CaCaM	16:46	0.2	OOS	47:20	0.1
LaLaCa	16:43	4.6	LLO	27:55	0.5	CaMO	24:32	0.2	MML	27:36	0.1
MMLa	26:01	4.3	MMCa	22:19	0.5	CyCyLa	11:40	0.2	MMCo	17:05	0.1
CyLaP	19:20	3.9	OOM	37:40	0.5	OOCy	23:34	0.2	MMS	41:44	0.1
LaMP	30:35	3.7	PPLa	36:22	0.5	MMM	30:40	0.2	LLS	34:14	0.1
LaLaO	24:32	2.8	POL	36:24	0.4	MPL	32:51	0.2	PPCy	26:20	0.1
CaLaP	36:16	1.9	MMP	36:22	0.4	MSO	44:46	0.2	CyPS	30:38	0.1
LaMO	28:47	1.8	PPO	44:40	0.4	MPS	46:23	0.2	CyCaO	16:13	0.1
LaLaS	30:38	1.3	LLL	23:10	0.4	PPM	41:58	0.2	PSL	43:32	0.1
CyLaO	18:18	1.2	LaPS	41:44	0.4	CaMS	30:38	0.2	LaSL	47:20	0.1
CyCaLa	13:04	1.1	LaOL	25:49	0.4	CyOL	19:05	0.1	CaCaO	18:18	0.1
LaPO	34:09	1.1	OOL	28:00	0.4	PPL	38:32	0.1	CySO	28:42	0.1
CyLaS	22:45	1.1	MMO	34:09	0.4	CaPL	28:33	0.1	MSL	37:40	0.1
LaMS	36:16	1.0	CyCaP	16:43	0.4	CyCaS	19:37	0.1	CyPL	20:34	0.1
CyMP	22:19	0.9	OOO	41:00	0.3	PSO	48:58	0.1	CoCaLa	11:37	0.1
MMCy	19:18	0.8	CyMO	21:03	0.3	CaLaO	20:51	0.1	CyLaL	15:12	0.1
CoLaM	14:49	0.8	LLP	29:38	0.3	OOCo	18:59	0.1	CaPS	36:16	0.1
CyCaM	14:52	0.7	LaSO	39:45	0.3	CoLaS	19:09	0.1	PPCa	30:38	0.1
OOLa	31:47	0.7	MOL	30:37	0.3	CyCyM	13:04	0.1	SSLa	46:34	0.1
CaLaO	20:51	0.7	LaML	23:38	0.3	OOCa	26:51	0.1			

[a]See "Experimental" for analysis conditions.
[b]Triacylglycerol fatty acids: capric (Ca), caprylic (Cy), caproic (Co), lauric (La), myristic (M), palmitic (P), oleic (O), linoleic (L), and stearic (S).
[c]Absolute retention time in minutes:seconds. See Figure 1 for coconut oil total mass spectrometric ionization chromatogram.
[d]Percent composition calculated by response factor to give adjusted composition (33).

The TAG names, with absolute retention time and corrected area percent composition in the order of decreasing composition for each TAG identified in the coconut oil sample, are given in Table 1. The coconut oil RP-HPLC/APCI-MS total ionization chromatogram is presented in Figure 1. Retention location of a particular coconut oil TAG along the chromatogram can be obtained by reference to the TAG retention time in Table 1. Ninety-nine TAGs, with corrected area percent of 0.1% or greater, were identified by RP-HPLC/APCI-MS for the coconut oil sample.

The most abundant TAGs greater than 5% were LaLaM (12.8%), followed in decreasing abundance order by LaLaLa (9.2%), LaLaCy (8.3%), CyLaM (7.5%), and CaLaM (5.6%). These TAGs accounted for 43.5% of the TAGs detected in coconut oil. Fourteen coconut oil TAGs were found at compositions between 1% and less than 5% to account for 34.4% of the identified TAGs in coconut oil. Thus, a total of 19 TAGs accounted for 77.4% of the coconut oil TAGs, which occurred at compositions of 1.0% or greater.

Fatty acid composition was calculated from the TAG composition as determined by APCI-MS and listed in Table 1. Similarly, FA composition determined by GC of the transmethylated coconut oil TAG is listed above. The FA composition calculated from RP-HPLC/APCI-MS compared to the FA composition determined as FAMEs by GC-FID shows a low average absolute error of 0.64%. Individual FA absolute errors are the following: Co (0.1%), Cy (1.5%), Ca (0.4%), La (2.2%), M (0.4%), P (0.2%), L (0.4%), O (0.5%), and S (0.1%). These

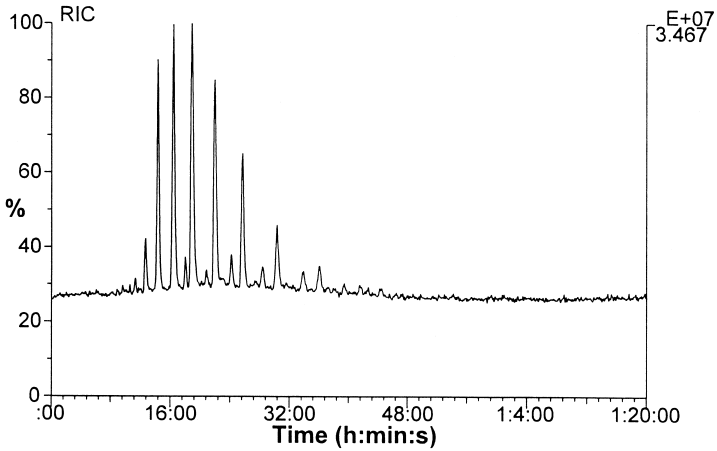

Figure 1. Reconstructed ion chromatogram (RIC) obtained by APCI-MS of coconut oil. See Table 1 for TAG chromatogram peak identification with respect to retention time. See "Experimental" for analysis conditions.

results confirm the identification and quantitation here of complex TAGs mixture, such as coconut oil TAGs.

Palm Oil

This oil contained 1.1% M, 47.2% P, 38.8% O, 8.9% L, and 3.9% S, as determined by calibrated GC of the FAMEs from the transmethylated oil. The TAG composition obtained by RP-HPLC/APCI-MS for palm oil is listed in Table 2. The corrected TAG composition obtained by RP-HPLC/APCI-MS showed 27 TAGs at 0.1% or greater. The most abundant TAGs at 5.0% or greater were PPO (35.0%), followed by OOP (24.2%), PPL (9.6%), PLO, (8.8%), and POS (5.5%) to account for 83.1% of the palm oil TAGs.

The FA composition, which was calculated from the TAG composition listed in Table 2 as determined by APCI-MS, is 1.0% M, 45.8 P, 9.3% L, 40.2% O, and 3.7% S. Comparison of this calculated FA composition with the experimental FA composition given above, determined by GC of the transmethylated palm oil, showed a low average absolute error of 0.7% with the values 0.1, 1.4, 0.2, 1.4, and 0.2% for M, P, L, O, and S, respectively. These results confirm the identification and quantitation of the palm oil TAGs.

Retention data for individual TAGs, for which the composition data are listed in Table 2, are given in the retention index values in Table 2. In the retention index file, mass spectrometric scan numbers are used instead of retention times because results can be given to two significant figures. All scan numbers are referenced to PPO because it was one of the most abundant TAGs for all the oils, except for coconut oil. This retention index procedure gave the lowest standard deviation (0.01–0.04) between all of the different runs of the different oil samples.

Randomized Palm Oil

This oil contained five FAs, which were 1.1% M, 45.7% P, 9.2% L, 39.8% O, and 4.2% S, as determined by calibrated GC of the FAMEs from the transmethylated oil. This oil contained, as expected, essentially the same FA composition as nonrandomized palm oil.

The TAG composition obtained by RP-HPLC/APCI-MS for randomized palm oil is listed with the TAG retention index in Table 2. The corrected experimental TAG composition obtained by RP-HPLC/APCI-MS detected 28 TAGs at 0.1% or greater. The most abundant TAGs PPO at 5.0% or greater were PPO (23.1%) followed by OOP (21.4%), PPP (10.8%), and OOO (7.9%) for a total of 63.2% randomized palm oil TAGs. These data, compared with the statistically

Table 2. TAGs Determined by RP-HPLC Coupled with APCI-MS[a]

		% Composition[d]						
			Randomized Palm Oil			Randomized Palm Olein		
TAG[b]	Retention Index[c]	Palm Oil	Exper.	Stat.	Palm Olein	Exper.	Stat.	Cocoa Butter
MMM	0.70	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MMP	0.83	0.1	0.1	0.0	0.1	0.1	0.0	0.0
MML	0.62	0.1	0.0	0.0	0.1	0.0	0.0	0.0
MMO	0.77	0.0	0.1	0.0	0.0	0.1	0.0	0.0
MMS	0.94	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MPL	0.75	0.3	0.2	0.3	0.4	0.2	0.3	0.0
MPO	0.89	1.4	1.1	1.2	1.3	0.9	1.2	0.0
MPS	1.05	0.1	0.1	0.1	0.1	0.1	0.1	0.0
MLO	0.70	0.1	0.2	0.2	0.2	0.3	0.4	0.0
MLS	0.86	0.0	0.0	0.0	0.0	0.1	0.0	0.0
MOS	1.00	0.1	0.1	0.1	0.2	0.1	0.1	0.0
PPP	1.05	1.2	10.8	9.5	0.8	7.6	6.6	0.1
PPM	0.70	0.3	0.6	0.7	0.2	0.4	0.6	0.0
PPL	0.86	9.6	6.0	5.7	9.8	6.1	5.7	2.0
PPO	1.00	35.0	23.1	24.8	29.9	20.9	20.0	17.9
PPS	1.16	0.3	2.6	2.6	0.2	2.4	2.2	0.4
PLO	0.81	8.8	10.0	10.0	10.6	11.6	11.6	0.9
PLS	0.95	1.3	1.0	1.0	1.5	1.2	1.3	3.2
POS	1.11	5.5	4.3	4.5	5.9	4.4	4.4	34.2
LLL	0.54	0.0	0.1	0.1	0.1	0.2	0.2	0.0
LLM	0.59	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LLP	0.68	1.9	1.2	1.2	2.6	1.9	1.6	0.2
LLO	0.64	0.5	1.0	1.0	0.7	1.9	1.7	0.1
LLS	0.79	0.2	0.2	0.1	0.4	0.4	0.2	0.1
LOS	0.92	0.7	0.8	0.9	1.1	1.2	1.3	1.0
OOO	0.90	3.8	7.9	6.3	4.7	8.0	6.9	0.9
OOM	0.84	0.4	0.5	0.5	0.5	0.4	0.6	0.0
OOP	0.96	24.2	21.4	21.6	22.8	20.6	20.3	5.8
OOL	0.77	1.6	4.1	4.4	2.3	6.0	5.9	0.2
OOS	1.06	2.1	1.7	2.0	2.8	2.1	2.2	8.0
SSS	1.36	0.0	0.0	0.0	0.0	0.0	0.0	0.3
SSM	1.15	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SSP	1.26	0.1	0.3	0.2	0.1	0.3	0.2	0.6

(continued)

Table 2. Continued

		% Composition[d]						
			Randomized Palm Oil			Randomized Palm Olein		
TAG[b]	Retention Index[c]	Palm Oil	Exper.	Stat.	Palm Olein	Exper.	Stat.	Cocoa Butter
SSL	1.06	0.1	0.1	0.1	0.2	0.1	0.1	2.0
SSO	1.21	0.3	0.3	0.2	0.4	0.3	0.2	22.0

[a]See "Experimental" for analysis conditions.
[b]Triacylglycerol fatty acids: myristic (M), palmitic (P), oleic (O), linoleic (L), and stearic (S).
[c]Retention index is the ratio of the mass spectrometric scan number for each TAG to the mass spectrometric scan number for PPO. Standard deviation: 0.01–0.04.
[d]Percent composition calculated by response factors to give adjusted composition (33). Exper., experimental; Stat., statistically calculated.

calculated TAG composition for randomized palm oil, show a low average absolute error of 0.3% for 26 predicted TAGs, which could be present at 0.1% or greater.

The calculated FA composition is 1.1% M, 45.5% P, 9.2% L, 40.1% O, and 4.1% S. These data compared with the experimental GC FA composition above had a low average absolute error of 0.1%. The absolute errors were 0.0, 0.2, 0.0, 0.3, and 0.1 for M, P, L, O, and S , respectively. These results confirm the identification and quantitation here of the randomized palm oil TAGs.

Palm Olein

This oil contained five FAs, which were 1.2% M, 42.0% P, 11.5% L, 40.9% O, and 4.5% S, as determined by calibrated GC of the FAMEs from the transmethylated oil.

The TAG composition obtained by RP-HPLC/APCI-MS for palm olein is listed in Table 2. The corrected TAG composition obtained by RP-HPLC/APCI-MS detected 28 TAGs at 0.1% or greater. The most abundant TAGs at 5.0% or greater were PPO (29.9%) followed by OOP (22.8%), PLO (10.6%), PPL (9.8%), and POS (5.9%) to account for 79.0% of the palm oil TAGs.

The FA composition, which was calculated from the TAG composition for palm olein listed in Table 2 as determined by APCI-MS is 1.2% M, 42.7% P,

11.3% L, 40.4% O, and 4.5% S. Comparison of this calculated FA composition with the experimental FA composition showed an average absolute error of 0.3%. Individual absolute errors for each FA were 0.0, 0.7, 0.3, 0.5, and 0.0% for M, P, L, O, and S, respectively. The low average absolute error, obtained between calculated and experimental FA compositions and low or no absolute error per FA, confirmed the accuracy of the TAG identification and quantitation obtained by APCI-MS for palm olein.

Randomized Palm Olein

This oil contained five FAs, which were 1.2% M, 41.1% P, 11.9 L, 41.1% O, and 4.6% S. The oil contained, as expected, essentially the same FA composition as nonrandomized palm olein.

For randomized palm olein, the corrected experimental TAG composition obtained by RP-HPLC/APCI-MS is listed with the TAG retention index in Table 2. The corrected TAG composition obtained by RP-HPLC/APCI-MS showed 29 TAGs. The most abundant TAGs at above 5.0% were PPO (20.9%), OOP (20.6%), PLO (11.6%), OOO (8.0%%), PPP (7.6%), PPL (6.1%), and OOL (6.0%), respectively, for a total 80.7% of the randomized palm olein TAGs. These data compared with the statistically calculated composition listed in Table 2 for randomized palm olein show a low average absolute error of 0.2% for 26 TAGs, which could be present at 0.1% or greater.

The FA composition calculated from the corrected TAG composition obtained by APCI-MS, compared to the experimental GC FA composition, had a low average absolute error of 0.1%. Absolute errors per specific FA were the following: 0.2% M, 0.1% P, 0.0% L, 0.2% O, and 0.0%, S, respectively. These accuracy results confirm the identification and quantitation of the TAGs in the palm olein sample.

Cocoa Butter

This oil contained five FAs, which were 0.1% M, 28.7% P, 3.4% L, 35.6% O, and 32.2% S, as determined by calibrated GC of the FAMEs from the transmethylated oil.

The TAG composition obtained by RP-HPLC/APCI-MS with the TAG retention index for cocoa butter is listed in Table 2. The corrected TAG composition obtained by RP-HPLC/APCI-MS showed 19 TAGs at 0.1% or greater. The most abundant TAGs at 5.0% or greater were POS (34.2%) followed by SSO (22.0%) and POP (17.9%). These three TAGs account for 74.1% of the cocoa

butter TAGs. The other TAGs at greater than 5.0% were SOO (8.0%) and OOP (5.8%) to account for 87.9% of the cocoa butter TAGs. The remaining 15 cocoa butter TAGs were detected at less than 5.0%.

The FA composition, which was calculated from the TAG composition listed in Table 2, as determined by APCI-MS, was 0.0% M, 28.7% P, 35.6% O, 3.4% L, and 32.3% S. Comparison of this calculated FA composition with the experimental FA composition determined by GC of the transmethylated cocoa butter TAGs given above produced a low average absolute error of 0.1%. Individual absolute errors were low for each FA, with the values 0.1, 0.0, 0.0, 0.0, and 0.1% for M, P, L, O, and S, respectively. The low average absolute error obtained between calculated and experimental FA composition and low or no absolute error per FA confirms the identification and quantitation of the cocoa butter TAGs.

TAG Physical Properties with Respect to TAG Composition

For food formulation products, what is usually the most important TAG designation with respect to food physical properties (such as melting point and solid fat content given in Table 3) is not so much the TAG species itself, but the food formulation TAG designation of saturated (S) and unsaturated (U) fatty acids (31). These data, along with total amount of saturated fatty acids, are listed for each oil in Table 4.

For example, concentrating on the 19 TAGs that occur at 0.1% or greater in coconut oil (after calculation from the TAG composition listed in Table 1), the

Table 3. Physical Properties of Triglycerides with High Amounts of Saturated Fatty Acids: Comparison of the Physical Properties of Coconut, Cocoa Butter, Palm Oil, and Palm Olein[a]

	Solid Fat Index[b]					
Oil	10.0°C	21.1°C	26.7°C	33.3°C	40.0°C	Melting point (°C)[b]
Randomized palm	32.3	21.3	19.3	14.5	9.9	41.8
Palm	33.5	14.1	11.2	8.7	4.9	39.2
Randomized palm olein	14.5	8.0	—	2.9	—	37.7
Cocoa butter	70.2	60.1	21.1	0.7	0.0	29.2
Coconut	53.6	25.5	0.0	0.0	0.0	26.6
Palm olein	19.0	5.2	—	0.6	—	21.7

[a]See "Experimental" for origin of fat samples.
[b]American Oil Chemist's Society method Cc 18-80 and Cd 10-57 (27).

Table 4. Component Glycerides of Food Oils[a] with High Amounts of Saturated Fatty Acids

	Component Glycerides (%)[b]				
Oil	UUU	UUS	USS	SSS	Saturated Acid (%)[c]
Radomized palm	13.1	36.0	36.3	14.5	51.0
Palm	5.9	38.4	53.6	2.1	52.2
Randomized palm olein	16.1	38.5	34.3	10.9	46.9
Cocoa butter	1.2	16.0	81.3	1.4	61.0
Coconut	1.6	3.9	10.5	84.0	92.3
Palm olein	7.8	41.0	49.5	1.4	47.7

[a]See Ref. 31.
[b]See Tables 1 and 2 for triglyceride species composition from which the summation of the unsaturated fatty acids (U) and saturated fatty acids (S) were calculated. The RP-HPLC and the APCI-MS methods (see "Experimental") do not readily distinguish between positional isomers. Thus, UUS also includes USU and USS includes SUS isomers.
[c]Total of the saturated fatty acids in the oil.

amounts of food formulation TAGs are 1.6% UUU, 3.9% UUS, 10.5% USS, and 84.0% SSS. For the TAG composition calculation of palm oil from TAGs listed in Table 2, the amounts of food formulation TAGs are 5.9% UUU, 38.4% UUS, 53.6% USS, and 2.1% SSS. Randomized palm oil has 13.1% UUU, 36.0% UUS, 36.3% USS, and 14.5% SSS.

The RP-HPLC method (see "Experimental"), unfortunately, will not resolve isomeric mono- and disaturated glycerides. Thus, the values for UUS include USU and USS including SUS. When considered as classes with respect to unsaturation, few differences exist, yet marked changes in physical properties, such as SFI and melting point are evident when fatty acids are moved about the triglyceride glycerol moiety during randomization.

Nevertheless, comparison of oils before and after randomization shows that changes in TAG UUU, UUS, USS, and SSS do occur in addition to changes in, for example, UUS and USU, the later of which we cannot detect here. Randomization of palm oil increased UUU and SSS TAGs and decreased USS TAGs with little change in UUS TAGs, compared to the nonrandomized palm oil. For randomized palm olein, the same TAG changes observed for randomized palm oil were observed for starting palm olein. Palm olein has 7.8% UUU, 41.0% UUS, 49.5% USS, and 1.4% SSS. In each case, the melting point and quantity of solids with increased temperature (SFI index) were greater after randomization of the oil. Some of this change can be attributed to changes in the glyceride components, which can be determined from TAG species quantitation by RP-HPLC/APCI-MS.

Reference to Tables 3 and 4 shows that the observed SFI and melting point for each oil are less related to the total saturated FA than to glyceride components. For example, coconut oil, which had the greatest amount of total fatty acids of the oils, had next to the lowest melting point. Also, coconut oil had no solids above 21.1. Randomized palm oil, with the balanced UUU = SSS to UUS = USS TAG composition, had the highest melting point and SFI of the oils studied. An increase in the SSS TAG may be, in part, responsible for lowering the melting point. Palm olein, which had the lowest melting point and SFI, had the highest amount of UUS TAG. Concentrations of UUU, UUS, USS, and SSS TAGs, which can be determined accurately from RP-HPLC/APCI-MS, are obviously important with regard to physical properties of food formulation fats.

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Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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