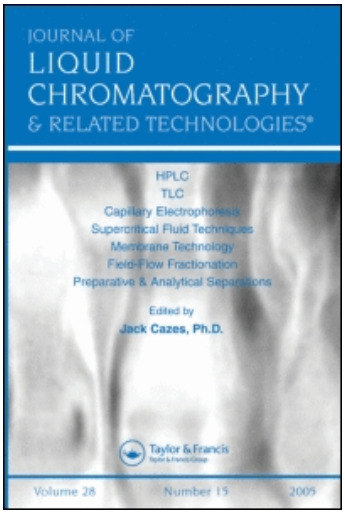

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 05 September 2002

To cite this Article Neff, William E. , Byrdwell, W. C. , Steidley, Kevin R. , List, Gary R. and Snowder, Gary(2002) 'TRIACYLGLYCEROL STRUCTURE OF ANIMAL TALLOWS, POTENTIAL FOOD FORMULATION FATS, BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY', Journal of Liquid Chromatography & Related Technologies, 25: 6, 985 — 998
To link to this Article: DOI: 10.1081/JLC-120003275
URL: http://dx.doi.org/10.1081/JLC-120003275

J. LIQ. CHROM. & REL. TECHNOL., 25(6), 985–998 (2002)

TRIACYLGLYCEROL STRUCTURE OF ANIMAL TALLOWS, POTENTIAL FOOD FORMULATION FATS, BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

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ABSTRACT

Triacylglycerol (TAG) compositions, as mole percent, were obtained by reverse phase high performance liquid chromatography (RP-HPLC), coupled with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) of lard and mutton tallow fractions under consideration for food formulation products. Accurate identification and quantitation of these TAG compositions were obtained and proved by comparison of the

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fatty acid composition calculated from the TAG composition which was obtained by APCI-MS with the fatty acid composition, and converted to mole percent as obtained by gas chromatography of the methyl esters of the transmethylated oils. Average absolute errors with respect to TAG quantitation and identification were less than one percent. Our study identified and quantitated the TAG equal to and greater than 0.1% concentration. Concentration of TAG with various fatty unsaturated (U) and saturated (S) fatty acids with TAG, designated as these species UUU, UUS, USS, and SSS, can be determined accurately from RP-HPLC/APCI-MS of the actual TAG species. These TAG species UUU, UUS, USS, and SSS affect the physical properties of food formulation fats.

INTRODUCTION

Improvement of the functional properties of food formulation 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 confectionary products, has been in progress. However, traditional fats such as vegetable oils, as coconut, cocoa butter, palm, and animal tallows remain important (10).

The triacylglycerol (TAG) composition (i.e., kinds and quantities of individual TAG) and TAG structure (i.e., kinds and quantities of individual FA 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. We are continuously investigating different fats as substitutes or improvements with better functional properties for presently important commercial fats, such as coconut and cocoa butter in confectionary and other food formulation products (16). Thus, it is important to know TAG composition of the these fats compared to the TAG composition of coconut and cocoa butter. In summary, the knowledge of the kinds and quantities of individual TAG in vegetable oils is important in food chemistry.

Previously, for qualitative TAG analysis, identification of TAG resolved by reversed 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 TAG (17–19). Recently, RP-HPLC coupled with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) has been utilized to conclusively identify eluting TAG during RP-HPLC of fats (20,21). The MS of the TAG through the utilization of the APCI-MS method gave spectra, which contained a simplified number of very distinctive fragment ions that included diacylglycerol, protonated molecular, and molecular related ions that conclusively identified individual TAG in vegetable oil TAG mixtures. Thus, this new methodology permitted the facile determination of the kinds of TAG in many vegetable oil mixtures (VGO).

For quantitative TAG analysis, the weakness has been the method of detection, since TAG do not have strongly chromatophoric groups, and the gradient solvent system needed for RP-HPLC TAG resolution present absorbance problems for the commonly used HPLC ultraviolet absorbance detector. Christie, and others have written extensive reviews on possible HPLC detectors for quantitative TAG analysis (22–27). The consensus of these authors is that, while not perfect, the HPLC flame ionization (FID) and evaporative light scattering (ELSD) detectors are the preferred detectors for quantitative TAG analysis. We have made extensive use of a FID for quantitative HPLC analysis of individual TAG 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 calculated from the experimental TAG composition against experimental FA composition obtained from GC analysis of the transmethylated VGO mixture.

We have also utilized the atmospheric pressure chemical ionization mass spectrometry (APCI-MS) as a quantitative detector for reversed phase HPLC of VGO (20,21). This detector was determined to give quantitative results for TAG, through facile calculation of individual response factors of individual TAG, based on the raw MS response and the FA composition previously obtained by GC of the transmethylated 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 TAG. Thus, reversed phase HPLC coupled with a quadrapole mass spectrometer through an atmospheric pressure chemical ionization source can more completely identify and quantitate all TAG present in a vegetable oil than other TAG identification techniques.

It is important to accurately know the TAG structure of fats in the development of food formulation products for confectionary, shortening, margarine, and other food products. In this report, the TAG compositions of

animal tallow were obtained by RP-HPLC coupled with the APCI-MS as a HPLC detector. Accurate identification and quantitation of these TAG compositions was 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.

EXPERIMENTAL

Materials

Mutton crude fats were supplied by G. Snowder of the Sheep Experiment Station, ARS, USDA, Dubois, ID 83423. The lard tallow was purchased locally from a commercial source. HPLC mobile solvents, acetonitrile (ACN) and dichloromethane (DCM), were HPLC grade and were purchased from EM Science (Gibbstown, NJ) and Fisher Scientific (Fair Lawn, NJ), respectively, and used without further purification.

High Performance Liquid Chromatography

The HPLC system used for RP-HPLC/APCI-MS contained a LDC 4100 MS, Thermo Separation Products, 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). 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) 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 liters per minute. 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). Injections of 10 mL were made using a Series 1050 autosampler, Hewlett-Packard (Wilmington, DE).

Atmospheric Pressure Chemical Ionization Mass Spectrometer Detector

Identification and quantitation of TAG components was performed with APCI-MS, which was a Finnigan MAT TSQ700 (San Jose, CA) 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, 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 to 2.0 sec. 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 (FAME) were prepared by the 0.5 N hydrochloric acid in methanol transmethylation of the TAG mixtures (28). The FAME were analyzed using calibrated gas chromatography (GC) according to this procedure. 0.5 μL FAME sample solution (5 mg sample per mL hexane) was analyzed by direct injection capillary GC. The capillary column was a SP2380 column, 30 m × 0.25 mm i.d. with 0.2 μm film thickness, Supelco, Inc. (Bellefonte, PA). The gas chromatograph was a Star model 3400 equipped with a flame ionization detector, Varian, Inc. (Walnut Creek, CA). 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 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°C and 280°C, respectively. The GC calibration mixture was FAME mixture 20 A, NU-CHEK-PREP, Inc. (Elysian, MN).

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

RESULTS AND DISCUSSION

TAG compositions by area percent were obtained by RP-HPLC/APCI-MS of highly saturated fatty acid fats such as lard, mutton fat, and a blend of lard and mutton fat. 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 confectionary, 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 (29). Identification of individual TAG 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 straightforward and facile. 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.

TAG identification from the APCI-MS spectra was based on mass spectra, which showed minimal fragmentation occurred (30). The fragmentation resulted primarily in diglyceride $[M\text{-}RCOO]^+$ ions and $[M + 1]$ protonated ions. The degree of fatty acid unsaturated in the TAG 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 ion as the abundant ions with diglyceride peaks representing 13 to 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 20 to 28% of the base peak. No $[M + 1]$ ions were found in the spectra of triglycerides which contained only saturated fatty acid, only the characteristic TAG identifying diglyceride pairs. Extracted ion chromatograms were utilized to identify those TAG, which had the same RP-HPLC retention.

For TAG quantitation via APCI-MS data, the following procedure was used for lard and sheep tallow. The total ionization chromatogram area counts for each TAG was obtained by the summation 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. TAG, which contain a high degree of unsaturation, produced more molecular ion and give less overall response, while those TAG which 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 TAG determined by APCI-MS was developed (31,32).

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 FA 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. When the full set of TAG response factors was applied to the raw APCI-MS data, the adjusted or corrected TAG area percent data resulted, for example the data given in Table 1. 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 (20,31,32). This method for TAG quantitative analysis is extended to the present study of the triglyceride compositions of food formulation products.

The TAG of the blend of 1 : 1 lard and sheep tallow required a different APCI-MS quantitation approach than the approach used for both the lard and sheep fat tallow (20,32). This different quantitation method called the "Blend Modification Method" is needed for accurate quantitation of the TAG in blends of fats, in which the TAG have not then interesterified. The procedure was extensively explained and demonstrated previously (32).

Accuracy of TAG identification and quantitation for each fat 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.

Retention data for individual TAG, for which the composition data are listed in Table 1, are given as the retention index file in Table 1. 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 TAG's for all the fats. This retention index procedure gave the lowest standard deviation (0.01 to 0.04) between all of the different runs of the different fat samples.

Lard

This fat contained eight FA, which were: myristic (M),C 14 : 0, 1.7%; palmitoleic (Po), C 16 : 1, 2.3%; palmitic (P), C 16 : 0, 26.4%; linoleic (L), C 18 : 2, 12.1%; oleic (O), C 18 : 1, 40.3%; stearic (S), C 18 : 0, 15.8%; erucic (Eu),

Table 1. TAGs Determined by RP-HPLC Coupled with APCI-Ms[a]

TAG[b]	Retention Index[c]	% Composition[d]		
		Lard	Sheep Fat	Lard : Sheep Fat (1 : 1)
MMP	0.83	0.0	0.2	0.1
MMO	0.77	0.1	0.2	0.1
MMS	0.94	0.0	0.1	0.1
MPL	0.75	0.2	0.1	0.2
MPO	0.89	0.7	1.8	1.3
MPS	1.05	0.3	1.9	1.0
MLO	0.70	0.8	0.1	0.5
MLS	0.86	0.3	0.1	0.2
MOS	1.00	0.8	1.6	1.2
PPP	1.05	0.3	1.1	0.6
PPM	0.70	0.1	0.8	0.4
PPL	0.86	1.9	0.2	0.9
PPO	1.00	6.1	6.5	5.8
PPS	1.16	2.5	4.3	3.9
PLO	0.81	11.8	0.9	6.2
PLS	0.95	3.3	0.7	2.0
POS	1.11	14.0	17.4	15.7
LLL	0.54	0.3	0.0	0.1
LLM	0.59	0.1	0.0	0.0
LLP	0.68	1.3	0.1	0.7
LLO	0.64	1.7	0.1	0.9
LLS	0.79	0.3	0.1	0.2
LOS	0.92	3.1	1.2	2.5
OOO	0.90	4.7	2.3	3.7
OOM	0.84	1.1	0.8	0.9
OOP	0.96	19.4	13.6	17.2
OOL	0.77	4.6	1.1	2.7
OOS	1.06	3.7	8.7	6.8
SSS	1.36	0.8	3.4	1.8
SSM	1.15	0.1	0.6	0.3
SSP	1.26	4.5	7.0	5.8
SSL	1.06	0.2	0.3	0.0
SSO	1.21	1.0	13.5	6.1
MPoP	0.79	0.0	0.2	0.1
MPoO	0.79	0.1	0.2	0.2
MPoS	0.90	0.0	0.2	0.1
PoPL	0.77	0.3	0.1	0.3
PoPO	0.87	1.5	1.6	1.3
PoPS	1.00	0.4	1.3	0.7
PoLL	0.65	0.2	0.0	0.1

Table 1. Continued

TAG[b]	Retention Index[c]	% Composition[d]		
		Lard	Sheep Fat	Lard : Sheep Fat (1 : 1)
PoLO	0.75	1.0	0.2	0.9
PoLS	0.85	0.3	0.1	0.2
PoOO	0.86	1.4	0.7	1.3
PoOS	0.98	1.4	2.0	1.7
PoSS	1.08	0.1	0.4	0.2
POEu	1.05	0.5	0.2	0.3
PSA	1.21	0.3	0.1	0.2
OOEu	1.03	0.3	0.1	0.1
LOEu	0.96	0.3	0.0	0.1
POA	1.15	0.2	0.1	0.1
PoLL	0.65	0.2	0.0	0.1
PSEu	1.12	0.1	0.2	0.2
PLEu	0.93	0.1	0.0	0.1
PoPP	0.90	0.1	0.3	0.2
PoSS	1.08	0.1	0.0	0.2
SSA	1.25	0.1	0.0	0.1
OSEu	1.12	0.1	0.2	0.2
LLEu	0.80	0.1	0.0	0.0
LOA	1.03	0.1	0.0	0.0
OOA	1.12	0.1	0.0	0.0
PPA	1.17	0.1	0.0	0.0
PLA	1.09	0.1	0.0	0.0
PoPoO	0.78	0.0	0.1	0.1
PPEu	1.07	0.0	0.1	0.1
OSA	1.20	0.0	0.1	0.1
SSEu	1.19	0.0	0.1	0.1
PoPoS	0.87	0.0	0.1	0.0
MOEu	1.11	0.0	0.0	0.1
TAG < 0.1%		0.3	0.1	0.6

[a]See "Experimental" for analysis conditions.
[b]Triacylglycerol fatty acids: myristic (M), palmitic (P), oleic (O), linoleic (L), stearic (S), palmitoleic (Po), erucic (Eu), and arachidic (A).
[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.

C 20 : 1, 0.6% and arachidic (A), C 20 : 0, 0.4% acids, as determined by calibrated GC of the FAME from the transmethylated oil.

The TAG names with relative retention data and corrected area percent composition equal to or greater than 0.1% for each TAG identified in the lard sample, are given in Table 1. Fifty-eight TAG with corrected area percent of 0.1% or greater, were identified by RP-HPLC/APCI-MS for the lard sample. The most abundant TAG greater than 5% were four TAG. These TAG were: POO (19.4%) followed in decreasing abundance order by POS (14.0%), PLO (11.8%) and PPO (6.1%). The above TAG accounted for 51.3% of the detected lard TAG. Sixteen lard TAG were found at composition of between 1 and less than 5% to account for 48.4% of the identified lard oil TAG. Thus, a total of twenty TAG accounted for 99.7% of the lard oil TAG, which occurred at composition greater than or equal to 1%. There are thirty-eight TAG less than 1% but greater than or equal to 0.1%. The remaining 0.3% of the TAG were detected at levels less than 0.1%. The TAG less than 0.1% included those TAG with linolenic acid. Thus, no TAG with linolenic acid are included in Table 1.

A fatty acid composition was calculated from the TAG composition as determined by APCI-MS listed in Table 1. Similarly, FA composition determined by GC of the transmethylated lard TAG is listed above. The FA composition calculated from RP-HPLC/APCI-MS compared to the FA composition determined as the FAME by GC-FID showed a low verage absolute error of 0.3%. Individual FA relative errors are the following: M, 0.0%; Po, 0.1%; P, 0.9%; Ln, 0.5%; L, 0.1%; O, 0.1%; S, 0.7%; Eu, 0.% and A, 0.0%. These results offer a high level of confidence with respect to the identification and quantitation, here, of a complex TAG mixture such as natural lard TAG.

Sheep Tallow

This fat contained M, 3.3%; Po, 2.7%, P, 24.0%; Ln, 1.0%, L, 2.0%; O, 36.2%; S, 30.4%; Eu, 0.4% and A, 0.1%, as determined by calibrated GC of the FAME from the transmethylated fat. The TAG composition obtained by RP-HPLC/APCI-MS for sheep fat is listed in Table 1. The corrected TAG composition obtained by RP-HPLC/APCI-MS, detected fifty-four TAG 0.1% or greater. The most abundant TAG at 5.0% or greater were six TAG: POS (17.4%) followed by POO (13.6%), SOS (13.5%), OOS (8.7%), PSS (7.0%), and POP (6.5%) to account for 66.7% of the sheep fat TAG. The number of TAG detected at levels 1 to 5% are twelve. Thus, a total of eighteen TAG greater than or equal to 1% accounted for 90.8% of the sheep fat TAG. Thirty-six TAG were detected at less than 1% but greater than or equal to 0.1%. The remaining combined TAG equaled 0.1%. These TAG were each detected at a level less than 0.1%.

The FA, which was calculated from the TAG composition listed in Table 1 as determined by APCI-MS is: M, 3.2%; Po, 2.6%; P, 24.9%; Ln, 0.0; L, 2.0%;

O, 35.5%; S, 31.4%; Eu, 0.3% and A, 0.1%. Comparison of this calculated FA, with the experimental FA composition given above determined by GC of the transmethylated sheep fat, showed a low average absolute error of 0.4%. The individual absolute error values are: M, 0.1%; Po, 0.1%; P, 0.9%; Ln, 1.0%; L, 0.0%; O, 0.7%; S, 1.0%; Eu, 0.0% and A, 0.0%.

These results offer a high level of confidence with respect to the identification and quantitation of the sheep fat TAG.

Blend 1 : 1 Lard : Sheep Tallow

This fat blend contained five FA, which were: M, 2.5%; Po, 2.5%; P, 25.2%; Ln, 0.8%; L, 7.1%; O, 38.2%; S, 23.1%; Eu, 0.5% and A, 0.3% as determined by calibrated GC of the FAME from the transmethylated fat.

The TAG composition obtained by RP-HPLC/APCI-MS for the blend is listed with TAG retention index listed in Table 1. The corrected, experimental TAG composition obtained by RP-HPLC/APCI-MS and corrected as previously described (32), detected 59 TAG 0.1% or greater. The most abundant TAG at 5.0% or greater were six TAG : POO (17.2%) followed by POS (15.7%), SOO (6.8%), PLO (6.2%), PPO (5.8%) and PSS (5.8%) for a total of 57.5% blend TAG. The number of TAG less than 5% but equal to or greater than 1% is twelve. These TAG represent 24.4% of the detected TAG. The remaining TAG less than 1%, but greater than or equal to 0.1% equaled 17.5% of the TAG. The detected TAG detected at less than 0.1% were 0.6% of the total blend TAG.

The calculated FA gave this composition: M, 2.5%; Po, 2.6%; P, 26.0; Ln, 0.0%; L, 7.2%; O, 38.2%, S, 22.8%; Eu, 0.5% and A, 0.3%. These data compared to the experimental GC FA above, had a low average absolute error of 0.2%. The individual absolute error values are: M, 0.0%; Po, 0.1%; P, 0.8%; Ln, 0.8%; L, 0.1%; O, 0.0%; S, 0.2%; Eu, 0.0% and A, 0.0%. These results give a high level of confidence with respect to the identification and quantitation, here, of the 1 : 1 lard : sheep fat TAG.

TAG Physical Properties with Respect to TAG Composition

For food formulation products, what is usually the most important TAG designation in respect to food physical properties, such as melting range and solid fat content is not so much the TAG species, but food formulation TAG designation per saturated (S) and unsaturated (U) fatty acids (11,33). For example, for lard tallow concentrating on the TAG, which occur at 0.1% or greater, after calculation from the TAG composition listed in Table 1, the amount of food formulation TAG are: UUU, 16.3%; UUS, 45.5%; USS, 29.7%; and SSS,

8.5%. For sheep tallow TAG composition calculation from TAG listed in Table 1, the amount of food formulation TAG are: UUU, 5.1%; UUS, 30.5%; USS, 45.0%; and SSS, 19.4%. For the blend TAG composition calculation from TAG listed in Table 1, the amount of food formulation TAG are: UUU, 10.2%; UUS, 38.5%; USS, 37.0%; and SSS, 14.3%. TAG composition is more related than, for example, total content of saturated fatty acid to food formulation product physical properties (11,33). It was previously observed that physical properties such as solid fat index (SFI) and melting point are less related to the total saturated FA than to glyceride components expressed as UUU, UUS, USS, and SSS (33). Concentration of UUU, UUS, USS, and SSS TAG, which can be determined accurately from RP-HPLC/APCI-MS is important in regard to physical properties of food formulation fats.

ACKNOWLEDGMENT

"Names are necessary to report factually on available data; however, the 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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Received November 19, 2001
Accepted December 13, 2001
Manuscript 5691