Triacylglycerol Analysis of Potential Margarine Base Stocks by High-Performance Liquid Chromatography with Atmospheric Pressure Chemical Ionization Mass Spectrometry and Flame Ionization Detection

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Several margarine base stock candidates have previously been prepared for the purpose of finding better, more oxidatively stable food components: high-saturate vegetable oils, randomized vegetable oils, vegetable oil−hard stock blends, and interesterified vegetable oil−hard stock blends. Here are reported the triacylglycerol compositions of these products, determined using reverse-phase high-performance liquid chromatography (HPLC) coupled with a flame ionization detector or a quadrupole mass spectrometer with an atmospheric pressure chemical ionization source. Triacylglycerol percent composition results for samples of known composition (randomized and interesterified samples) exhibited less average error by HPLC coupled with a quadrupole mass spectrometer with an atmospheric pressure chemical ionization source, after application of response factors, than the results by HPLC coupled with a flame ionization detector. The fatty acid compositions calculated from the mass spectrometric data exhibited less average error than the fatty acid compositions resulting from the flame ionization detector data. The average error of the fatty acid compositions by the mass spectrometer was lowest for interesterified blend samples, next lowest for randomized samples, then followed by high-saturated fatty acid oils, normal oils, and blends. Analysis of the vegetable oil−hard stock blends by mass spectrometer required special treatment for calculation of response factors.

Keywords: *Atmospheric pressure chemical ionization mass spectrometry; flame ionization detector; margarine base stocks; triacylglycerol analysis; triglyceride; triacylglycerol*

INTRODUCTION

The triacylglycerol (TAG) fraction of a margarine or shortening, called the base stock, is responsible for most of a product's physical properties, from texture to taste. Research has been directed toward improvement, through plant genetic manipulation, of the functional properties of vegetable oils (*1*−*9*), which may then be used as base stocks or components in base stocks (*10*, *11*). Improvements have been accomplished by alteration of the fatty acid (FA) composition and TAG composition, resulting in oils with wide-ranging compositions. Alternatively, base stocks have been prepared from either the randomization of a single oil, the blending of a traditional vegetable oil with hard stocks (TAG mixtures containing mostly trisaturates), or the interesterification of the vegetable oil with hard stocks blend. Vegetable oil blends with hard stocks produce TAG mixtures that exhibit more solids at higher temperatures and are less susceptible to oxidative degradation (*12*, *13*) than normal vegetable oil. Interesterification after blending, which may be chemically (*14*) or enzymatically (*15*) catalyzed, changes the TAG composition even further to provide improved food components (*16*, *17*).

The ability to characterize the TAG composition of a potential fat or oil food product is integral to the formulation of a desirable end product. Reverse-phase high-performance liquid chromatography (RP-HPLC) with a detector such as a flame ionization detector (FID) (*14*, *18*−*20*), refractive index (*18*), or evaporative light scattering detector (*20*) has become common for TAG analysis. These detectors have previously been shown to provide satisfactory qualitative and quantitative composition information for TAGs from a variety of sources. Quantification of TAG species using a FID has advantages over the other two-dimensional detectors in that it is linear over a broad range without the need for response factors (*14*, *18*, *19*), and it is not adversely affected by gradient runs. These various detectors have proven to be satisfactory for TAG analyses in which chromatographic standards were available for the TAG or in which structures could be identified by theoretical carbon number (*14*, *18*) or equivalent carbon length. Unfortunately, however, these detectors do not allow characterization of unidentified TAGs for which no standards are available, and they cannot differentiate between TAG species that are completely chromatographically overlapped. Sometimes, the failure to resolve overlapped peaks can cause some TAG species to be overestimated while others go unidentified. As more is learned about how specific molecular species affect the lubricity (texture, mouthfeel) and other character-

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istics of a fat or oil (*21*−*23*), it becomes increasingly important to be able to accurately determine the percentage composition of all individual TAG species.

Recently, we have demonstrated that, in the cases of normal (*24*−*27*) and genetically modified seed oils (*26*) and of synthetically useful seed oils (*24*, *25*), for which no commercial standards were available, RP-HPLC coupled to a mass spectrometer via an atmospheric pressure chemical ionization (APCI) source could provide excellent qualitative identification of molecular species and differentiation, by mass, of completely overlapped species. Others have demonstrated similar success with qualitative identification of TAG species in complex mixtures using APCI−mass spectrometry (MS) with silver ion LC (*28*) or supercritical fluid chromatography (*29*). Furthermore, we have shown that response factors may be calculated very easily, which allows quantification of TAG species (*26*, *27*, *29*). Quantification of TAGs from data obtained by RP-HPLC/APCI-MS has been shown to exhibit less error in the TAG compositions than corresponding FID data, compared to theoretically expected compositions. RP-HPLC/APCI-MS data also exhibited less error than LC-FID data in the FA compositions calculated from the TAG compositions, when compared to the FA compositions determined from the FA methyl esters, analyzed by GC-FID. Given the demonstrated effectiveness of RP-HPLC/APCI-MS for TAG analysis, we report now the extension of this methodology to the analysis of potential margarine base stocks made from vegetable oils, vegetable oil blends with hard stocks, and high stearic acid genetically modified oils.

We present in this study the RP-HPLC/APCI-MS compositional analysis of TAG species in the following margarine base stock samples: normal and randomized corn, soybean, and canola oils, respectively; high stearic acid genetically modified soybean oil samples; blends made from 80% (by wt) normal vegetable oil with 20% hard stocks; and interesterified vegetable oil with hard stock blends. These are samples for which we have already accumulated a significant amount of data using other techniques (*10*, *11*, *14*). By applying the newest methodology to samples for which we already have solid-fat index, dropping point, crystal structure, RP-HPLC-FID, and silver ion HPLC-FID data, we increase the amount of information available that can help to correlate TAG composition to functional properties. Also, although the method developed by us for quantification of TAGs works very well for most samples (*27*, *30*), the vegetable oil with hard stock blends present special complications that make it necessary to more thoroughly elucidate the strengths and limitations of the method.

MATERIALS AND METHODS

Materials. High-stearic soybeans coded HS-1 were supplied by the Jacob Hartz Seed Co. (Stuttgart, AR) from the 1993 crop year. High-stearic soybeans coded A-6 from the 1989 crop were supplied by Iowa State University (Ames, IA). These samples were processed using a hexane extraction method that has been previously described (*31*). Commercially refined, bleached, and deodorized normal corn, canola, and soybean oils were obtained from P.V.O. Foods (Granite City, IL). Fully saturated cottonseed oil saturates (cottonseed oil stearine) and soybean oil saturates (soybean oil stearine) flakes (hard stocks) were commercial preparations obtained from Riceland Foods (Stuttgart, AR) or Bunge Foods (Bradley, IL). All solvents were of HPLC grade or the highest available quality and used without further purification.

Blends, Interesterification, and Randomization of Vegetable Oil Samples. Liquid oils and hard stocks were blended and dried by heating under a water aspirator vacuum. Three blends were produced: normal corn oil−cottonseed oil stearine (80:20), normal soybean oil−cottonseed oil stearine (80:20), and normal canola oil−soybean oil stearine (80:20). Interesterification and randomization in the presence of sodium methoxide as catalyst were performed as previously described (*32*).

HPLC. For RP-HPLC/APCI-MS, a Thermo Separation Products (Shaumburg, IL) LDC 4100 MS quaternary pump system with membrane degasser was used. The columns used were two Inertsil ODS-80A (GL Sciences, Keystone Scientific, Bellefonte Park, PA), 25 cm × 4.6 mm, 5 μm, in series. The gradient elution was as follows: 70% acetonitrile, 30% dichloromethane held for 40 min; to 65% acetonitrile, 35% dichloromethane at 45 min, held until 55 min; to 60% acetonitrile, 40% dichloromethane at 60 min, held until 70 min; to 55% acetonitrile, 45% dichloromethane at 80 min. The flow rate was 0.85 mL/min throughout. The column effluent was split so that ∼720 μL/min went to an evaporative light scattering detector and ∼130 μL/min went to the APCI interface. A shorter gradient than that used for RP-HPLC-FID had to be used for RP-HPLC/APCI-MS because the MS data acquisition software (or the pump's firmware) limited RP-HPLC runs to 99.9 min. For RP-HPLC-FID, the pump used was a Thermo Separation Products SP 8800 system. A linear gradient solvent program from 60% acetonitrile/40% dichloromethane to 30% acetonitrile/70% dichloromethane, as described previously (*12*), was used. Ten microliters of a 25 μg/μL sample in dichloromethane was injected. The FID was a Tracor model 945 HPLC detector (Finnigan, Inc., Austin, TX). The FID operating conditions were as follows: block temperature of 130 °C, 140 mL/min hydrogen detector gas, 250 mL/min hydrogen cleaning flame gas, 175 mL/min oxygen, and 0.4 cubic ft/min air. FID data output was integrated by a mainframe computer system (*33*). TAG quantitation was obtained from chromatogram peak area percent without response factors. Area percent precision ranged from 0.0 to 0.7%.

MS. A Finnigan MAT (San Jose, CA) SSQ 710C quadrupole mass spectrometer fitted with an APCI source was used to acquire mass spectral data. Conditions have been described previously (*27*).

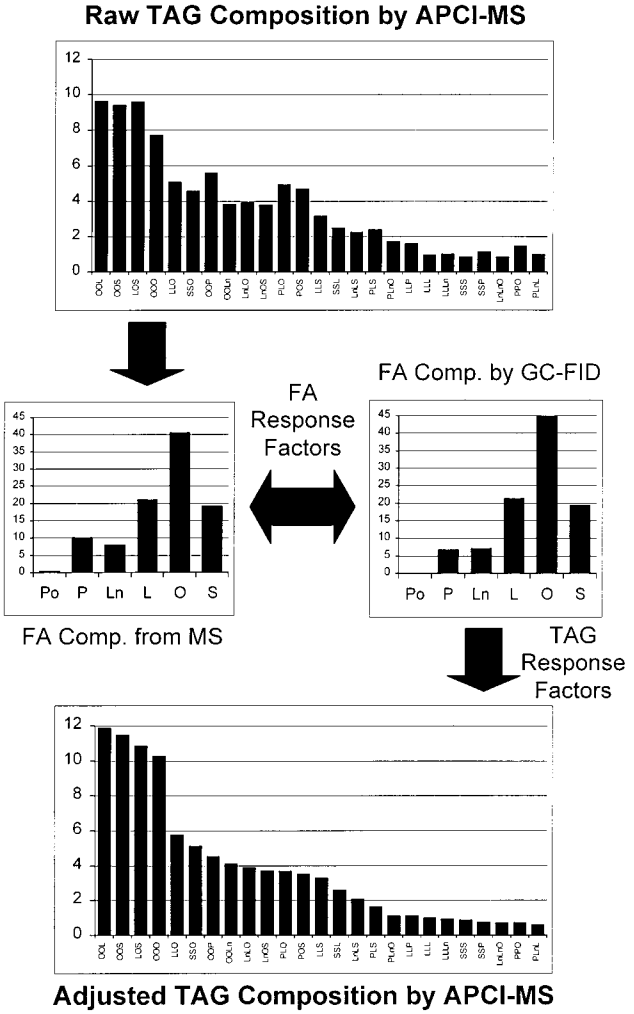
GC. FA methyl esters were prepared by the potassium hydroxide catalyzed transmethylation of the TAG mixtures (*19*).The FA methyl esters were analyzed using calibrated GC-FID according to this procedure. The sample solution, 5 μL (5 mg of sample/mL of hexane) was analyzed by direct injection capillary GC. The capillary column was an 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 an FID (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 as follows: 150 °C hold for 35 min, 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. The GC calibration mixture was an FA methyl ester mixture 20 A from Nu-Chek−Prep, Inc. (Elysian, MN).

Response Factor Calculation. Response factors were calculated for the corn oil TAGs, for example, by calculating the ratio of the FA composition by GC to the FA composition calculated from the raw TAG composition obtained by APCI-MS.

$$\mathbf{r}_{FA} = (FA\ \%_{GC-FID})/(FA\ \%_{APCI-MS})$$

This was then normalized to one of the fatty acids set equal to 1.0, which was usually the fatty acid with the least area percent (unless it was present at a very low level, in which case the fatty acid with the smallest area percent >1% was used). The final FA response factor, $\mathbf{R}_{FA}$, then equaled the

Scheme 1



initial response factor divided by the smallest response factor:

$$\mathbf{R}_{FA} = \mathbf{r}_{FA}/\mathbf{r}_{minimum}$$

The following table illustrates the response factor calculations for normal corn oil.

fatty acid	raw APCI-MS % composition	GC % composition	ratio $\mathbf{r_{FA}}$	response factor $\mathbf{R_{FA}}$
palmitic	15.61	11.69	0.7489	1.1178
linolenic	1.11	0.89	0.8018	1.1967
linoleic	51.73	60.19	1.1635	1.7366
oleic	28.56	25.21	0.8827	1.3175
stearic	3.00	2.01	0.6700	1.0000
minimum			0.670	

TAG response factors were calculated by multiplying the FA response factors together, as follows:

$$\mathbf{R}_{TAG} = \mathbf{R}_{FA1}\mathbf{R}_{FA2}\mathbf{R}_{FA3}$$

For example, the TAG response factor for tristearin was 1.0000, whereas that of dioleoylpalmitoylglycerol was 1.9403. The process of calculation of FA response factors followed by calculation of TAG response factors and then application to raw MS data is shown in Scheme 1, using the interesterified canola oil blend as an example. In practice, the FA percentage composition values were placed into an Excel spreadsheet and all resultant values were calculated using the appropriate equations. Calculated response factors were multiplied by the integrated peak areas to produce adjusted peak areas, which were then used to produce an adjusted TAG percent composition. Rounding to the appropriate decimal place was performed at the end, for the final adjusted TAG composition.

RESULTS AND DISCUSSION

Normal TAG Quantitation Method. TAG compositions by area percent were calculated from RP-HPLC/APCI-MS and RP-HPLC-FID data. FID data have been reported to be quantitative without the use of response factors (*14*). For APCI-MS data, the area 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. 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 produce more molecular ion and give less overall response, whereas those TAGs that are more saturated give mostly $[M - RCOO]^+$ fragments and larger peak areas. The saturates tend to be over-represented in percent compositions, whereas the unsaturates tend to be underrepresented. To solve this, a method for the calculation of response factors for TAGs determined by APCI-MS was developed. We have previously shown that the method developed produced less error, compared to known compositions, than did results by RP-HPLC-FID (*26*). This method for quantitative analysis is extended to the present study.

TAG compositions obtained by RP-HPLC/APCI-MS and by RP-HPLC-FID are presented in Tables 1−4 for corn oil, soybean oil, canola oil, and genetically modified soybean oil, respectively. Tables 5−8 give the FA compositions calculated from each of the TAG compositions, respectively, along with the FA composition determined as the FA methyl esters using calibrated GC-FID. Table 1 lists the TAG compositions for corn oil, randomized corn oil, the corn oil with hard stocks blend, and the interesterified corn oil with hard stocks blend. When the full set of TAG response factors was applied to the raw APCI-MS data, the adjusted area percent given in Table 1 resulted. The FA composition, which is calculated from the TAG composition determined by APCI-MS, is given in Table 5 and exhibits less average absolute error than the FA composition calculated from the TAG composition obtained by RP-HPLC-FID compared to the FID composition determined as the FA methyl esters. The process of calculation of response factors, and their application to the raw TAG composition determined by APCI-MS, is shown in Scheme 1, using data from the interesterified canola oil blend.

Throughout all margarine base stock samples, it is seen that for all normal, interesterified, and interesterified blend samples, the FA compositions obtained from the adjusted APCI-MS TAG compositions exhibited less average absolute error than the FA composition calculated from the RP-HPLC-FID TAG composition. Also, the FA compositions of the interesterified samples consistently exhibited less average absolute error than the normal oil samples. Furthermore, the TAG compositions obtained by APCI-MS for the randomized or interesterified samples agreed better with their statistically predicted compositions than did the TAG compositions obtained by RP-HPLC-FID. For randomized corn

Table 1. TAG Composition (Percent) of Corn Oil (CO) Margarine Base Stock by RP-HPLC with MS Detection and FID[a]

	CO		randomized CO			blend		interesterified blend		
TAG[b]	MS[c]	FID	MS	FID	PRE[d]	MS[e]	FID	MS	FID	PRE
LLO	21.5	23.0	22.9	24.9	27.2	18.8	17.4	14.4	15.4	15.0
LLL	25.4	22.6	22.3	21.5	20.1	22.2	19.5	12.3	11.5	11.4
LLP	14.7	15.2	14.2	14.2	12.5	11.2	14.0	9.8	10.1	9.8
OOL	10.7	10.6	12.4	10.7	12.3	9.0	8.1	7.3	6.2	6.6
PLO	10.0	10.4	10.0	10.4	11.2	7.6	9.0	8.0	10.2	8.6
PPL	2.5	1.7	2.6	2.1	2.6	1.7	1.8	3.0	2.6	2.8
OOP	2.9	2.4	3.1	2.1	2.5	2.6	2.3	1.9	1.9	1.9
LLS	2.2	1.8	1.8	1.8	2.2	0.8	1.6	11.0	10.2	11.1
LOS	1.8	1.3	2.1	1.4	2.0	0.6	1.3	8.7	9.0	9.7
OOO	2.8	3.2	2.9	2.8	1.8	2.9	2.3	1.2	1.3	1.0
PPO	0.9	0.4	1.1	0.6	1.2	0.8	0.3	1.3	1.0	1.2
PLS	0.8	0.4	0.9	0.5	0.9	0.3	0.4	5.9	5.9	6.4
LLLn	1.2	0.8	0.9	1.1	0.8	0.3	0.6	1.1	0.4	0.5
LnLO	0.9	2.3	0.9	2.0	0.7	0.3	0.2	0.1	1.2	0.4
OOS	0.6	0.5	0.6	0.4	0.4	0.3	0.4	2.2	1.6	2.1
POS	0.3	0.3	0.4	0.3	0.4	0.2	0.2	2.7	2.5	2.8
PLnL	0.5	0.5	0.4	0.6	0.3	0.2	0.1	0.6	0.6	0.3
PPP	0.0	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.4	0.3
OOLn	0.1	1.0	0.1	1.1	0.2	0.0	0.0	0.0	0.8	0.1
PLnO	0.1	0.5	0.1	0.8	0.2	0.0	0.0	0.1	0.0	0.1
PPS	0.0	0.1	0.1	0.1	0.1	2.6	2.3	1.0	0.7	0.9
SSL	0.1	0.3	0.1	0.2	0.1	0.0	0.1	3.5	3.3	3.6
LnLS	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.3
SSO	0.0	0.0	0.1	0.1	0.0	0.0	0.0	1.8	1.4	1.6
PPLn	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.0
SSP	0.0	0.1	0.0	0.0	0.0	7.4	7.3	1.2	1.0	1.0
SSS	0.0	0.1	0.0	0.0	0.0	10.0	10.3	0.7	0.6	0.4
sum[f]	100.1	99.8	100.2	100.0	100.0	100.0	99.7	100.3	99.9	99.9

[a] Species identified by MS at <0.05% and not detected by FID were omitted; other 0.0% in MS column indicate <0.05%. [b] TAG fatty acids: Ln, linolenic; L, linoleic; O, oleic; S, stearic; P, palmitic. [c] LC-MS data have been adjusted using response factors calculated using GC-FID data. See ref *26*. [d] Predicted TAG composition from statistical distribution of FA among the glycerol moiety carbons during chemical randomization or interesterification. [e] Calculated using two sets of response factors, one set for the base oil and one set for the hardstock TAGs—PPP, PPS, SSP, SSS. This method was used for all blend APCI-MS data in following tables. [f] Calculated at more decimal places, difference from 100% represents the sum of rounding errors.

oil, the average absolute error for the TAG composition obtained by LC-APCI-MS was 0.45%, whereas for the composition obtained by RP-HPLC-FID, it was 0.56%. For the interesterified corn oil blend, the average absolute error for the TAG composition obtained by RP-HPLC/APCI-MS was 0.27%, whereas for the composition obtained by RP-HPLC-FID, it was 0.35%. These corn oil samples exhibited the least dramatic differences between RP-HPLC/APCI-MS and FID results of any base stock samples. The interesterified soybean and canola blends produce an average absolute error for RP-HPLC/APCI-MS results, which was much less than that for the FID results. The smaller difference between APCI-MS and FID results for corn oil is likely because corn oil has fewer molecular species for a simpler TAG composition, due to the small amount of linolenic acid present. Although there are fewer molecular species (specifically linolenic-containing species) that may overlap with other molecular species in corn oil, the RP-HPLC-FID nevertheless suffers from an inability to distinguish between overlapped TAGs. For example, the FID results for the TAGs linolenoyllinoleoyloleoylglycerol, dioleoyllinolenoylglycerol, and palmitoyloleoyllinolenoylglycerol indicate a higher proportion of these species than do the results obtained by RP-HPLC/APCI-MS for normal corn oil, randomized corn oil, and the interesterified corn oil blend. The problem arises because these three TAGs occur only as shoulders on larger peaks in the RP-HPLC-FID chromatogram (linolenoyllinoleoyloleoylglycerol is a shoulder on trilinolein; dioleoyllinolenoylglycerol is a shoulder on dilinoleoyloleoylglycerol; palmitoyloleoyllinolenoylglycerol is a shoulder on dilinoleoylpalmitoylglycerol), and their peak areas include areas attributable to the overlapping TAGs. As the APCI-MS results show, these species are easily differentiated by the masses of their different fragments, so the TAG compositions by APCI-MS are very close to the predicted compositions for these samples.

TAG Quantitation Method for Oil Blends. The method developed by us for the quantification of TAGs, using response factors calculated from MS and GC-FID data, has been demonstrated to give better agreement to theoretical compositions of randomized fats and oils than FID and so has been used for normal and interesterified vegetable oils. For these vegetable oils, the method has produced good agreement of the TAG FA compositions by MS with the FA composition obtained by GC-FID. The method continues to work well for samples in which the FA profile in the extracted ion chromatograms is reflective of the overall FA profile. That is to say, if most of the extracted ion chromatograms show a small peak for stearic acid, then the FA composition reflects a small amount of stearic acid. The case of the non-interesterified blends is somewhat different. The method of calculation of response factors developed by us applies FA response factors evenly to all TAGs containing a particular fatty acid. The calculation method makes the inherent assumption that the over- or under-response of a fatty acid results from all of the TAGs which contain that fatty acid. Oil blends with hard stocks do not obey this assumption. In vegetable oil with hard stocks blends, a few specific TAGs contributed almost all of the stearic acid and much of the palmitic acid present. Thus, most extracted ion chromatograms showed a small amount of stearic

Table 2. TAG Composition (Percent) of Soybean Oil (SBO) Margarine Base Stock by RP-HPLC with MS Detection and FID[a]

	SBO		randomized SBO			blend		interesterified blend		
TAG	MS	FID	MS	FID	PRE	MS	FID	MS	FID	PRE
LLO	17.7	17.2	19.9	20.9	20.6	14.0	12.9	11.4	10.8	11.6
LLL	13.6	17.5	13.2	14.5	14.7	17.0	12.2	8.9	8.3	8.1
LLP	11.8	12.9	10.4	10.5	9.7	8.6	9.6	6.9	10.2	7.2
OOL	8.5	7.1	8.8	9.3	9.6	6.9	6.8	5.3	4.4	5.5
PLO	8.0	8.7	9.3	9.6	9.1	6.2	7.6	6.4	8.9	6.9
LLLn	6.7	7.2	6.8	4.9	5.9	5.1	4.8	3.3	2.3	3.2
LnLO	6.3	6.9	5.8	6.9	5.5	4.0	4.7	2.8	2.5	3.0
LLS	4.9	3.1	4.0	2.8	3.3	1.8	2.6	10.9	10.7	9.9
LOS	2.9	2.2	2.9	2.6	3.1	1.3	2.7	8.2	9.7	9.5
PLnL	2.5	3.2	2.3	2.7	2.6	1.9	2.5	1.8	2.1	1.9
PPL	2.4	1.7	2.4	1.8	2.2	1.6	1.6	2.2	3.1	2.1
OOP	2.5	1.7	2.2	1.7	2.1	2.1	2.1	1.6	1.5	1.6
OOO	2.5	2.2	1.8	1.7	1.5	2.8	2.4	0.9	0.8	0.9
PLS	1.6	0.9	1.5	1.0	1.5	0.5	1.2	5.7	6.3	5.9
OOLn	1.2	1.5	1.3	1.9	1.3	1.2	1.3	1.0	0.8	0.7
PLnO	1.5	1.7	1.3	1.7	1.2	0.8	1.2	1.0	1.4	0.9
PPO	0.6	0.5	1.0	0.7	1.0	0.5	0.5	1.1	1.1	1.0
LnLS	0.8	0.0	0.8	0.0	0.9	0.5	0.0	2.1	0.0	2.6
LnLnL	0.8	1.0	0.8	0.7	0.8	0.7	0.8	0.5	1.0	0.4
OOS	1.0	0.6	0.8	0.6	0.7	0.5	0.8	2.5	1.8	2.3
POS	0.5	0.3	0.6	0.6	0.7	0.2	0.4	2.9	2.9	2.8
LnOS	0.4	0.0	0.4	0.0	0.4	0.3	0.0	1.1	0.0	1.2
LnLnO	0.4	0.5	0.4	1.1	0.4	0.5	0.5	0.4	0.1	0.2
PPLn	0.2	0.1	0.2	0.2	0.3	0.1	0.2	0.3	0.3	0.3
SSL	0.4	0.3	0.3	0.2	0.2	0.1	0.3	4.4	3.7	4.1
PLnS	0.1	0.0	0.1	0.0	0.2	0.1	0.0	0.6	0.0	0.8
LnLnP	0.1	0.3	0.1	0.3	0.2	0.1	0.3	0.1	0.1	0.1
PPS	0.0	0.1	0.1	0.2	0.2	2.8	2.1	0.9	1.7	0.9
PPP	0.0	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.4	0.2
SSO	0.1	0.2	0.1	0.2	0.1	0.0	0.3	2.2	1.2	1.9
LnLnS	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2
SSP	0.0	0.1	0.0	0.2	0.1	7.3	7.0	1.2	0.9	1.2
SSLn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.5
SSS	0.0	0.1	0.0	0.1	0.0	10.0	10.3	0.7	0.9	0.6
sum	100.1	99.9	99.7	99.8	100.4	99.6	99.9	99.9	99.9	100.2

[a] See footnotes in Table 1 for definitions and experimental details.

acid, but the FA composition showed a high level of stearic. Almost all of the stearic acid came from three or four primary hard stock TAGs. Therefore, to apply response factors resulting from these saturates to all TAG species in the blend containing palmitic and stearic acids was not appropriate. Instead, a modification to the original method was developed to allow us to apply one set of response factors to the base oil, which constituted 80% of the weight of the blend and contained little stearic acid, and a different set of response factors to the three or four main hard stock TAGs. When combined, the two sets of response factor adjusted areas, representing 80 and 20% of the total, gave good agreement between the FA composition calculated from the TAG area percent with the FA composition determined by GC-FID. Using the canola oil/soybean oil stearine blend as an example, the method for calculation of response factors for a blend is demonstrated below.

The "Blend Modification" Method. The modification of our original quantitation method applicable to non-interesterified blends is shown in Scheme 2, using the canola oil blend data as an example. An overview of the process is as follows: First, the areas attributed to dipalmitoylstearoylglycerol (PPS), distearoylpalmitoylglycerol (SSP), and tristearin (SSS), which arose almost entirely from the hard stock, were subtracted from the TAG area composition. Then, the normal amounts of dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin to be expected in the base oil (very small amounts) were estimated from average peak sizes of palmitic and stearic in extracted ion chromatograms and added back in. The resultant TAG composition was referred to as the "hard stock-subtracted TAG composition". Because saturated TAG hard stocks made up 20% of the mixture, the palmitic and stearic acids in the hard stock represented 20% of the total FA composition by GC-FID. Therefore, 20% of the total FA weight was subtracted from the percentages of palmitic and stearic acids, and the remaining FA composition was recalculated to equal 100%; this was referred to as the "hard stock-subtracted GC-FID FA composition". The 20% was subtracted from the palmitic and stearic percentages in the FA composition in the proportion of the hard stock's known FA composition [10% palmitic and 90% stearic; see List et al. (*13*)]. Next, the FA composition of the hard stock-subtracted TAG composition was calculated and was used with the hard stock-subtracted FA composition by GC-FID to produce response factors for the base oil. Similarly, the areas of dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin, attributed to the hard stock, which were subtracted from the total area for the blend, were corrected by using response factors calculated from the GC-FID FA composition of the hardstock (10% palmitic and 90% stearic) and the FA composition calculated from the TAG area percent for the three TAGs. After the response factors were applied separately, the adjusted hard stock composition was then added back together with the adjusted base oil composition in a proportion of 20% hard stock and 80% base oil. The final

Table 3. TAG Composition (Percent) of Canola Oil (CNO) Margarine Base Stock by RP-HPLC with MS Detection and FID[a]

	CNO		blend			interesterified blend		
TAG	MS	FID	MS1[b]	MS2[c]	FID	MS	FID	PRE
OOO	23.8	23.5	13.6	17.2	16.1	10.0	8.0	8.8
OOL	18.5	19.4	15.4	19.6	15.7	11.7	10.3	12.7
OOLn	14.1	16.3	4.8	6.2	7.6	4.1	4.3	4.3
LnLO	9.9	9.1	2.9	3.7	6.1	3.9	4.4	4.1
LLO	7.9	4.5	5.5	7.1	5.4	5.7	4.5	6.1
LnLnO	4.3	3.7	0.8	1.0	1.5	0.7	0.9	0.7
OOP	4.1	4.8	4.2	5.1	4.1	4.9	4.8	4.5
PLO	3.6	5.2	3.9	4.7	5.4	4.0	8.3	4.3
PLnO	2.4	3.1	1.3	1.5	2.5	1.2	0.7	1.5
LLLn	2.1	0.9	1.0	1.2	0.9	0.9	0.9	1.0
OOS	1.6	1.1	2.2	1.1	0.9	11.2	11.6	11.4
PLnL	1.3	1.3	0.7	0.9	1.7	0.7	1.4	0.7
LOS	1.2	1.5	2.4	1.2	2.0	10.6	11.5	11.0
LLP	0.7	1.4	1.9	2.3	2.9	1.2	5.7	1.0
LnOS	0.6	0.0	1.8	0.9	0.0	3.7	0.0	3.7
LLL	0.6	0.7	1.8	2.4	1.3	1.0	0.5	1.0
LnLnP	0.5	0.0	0.2	0.2	0.4	0.2	0.2	0.1
PoPoLn	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LnLS	0.3	0.0	1.1	0.5	0.0	2.1	0.0	1.8
PoPO	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0
PPL	0.2	0.2	0.5	0.5	0.2	0.4	0.7	0.4
PPO	0.2	0.0	0.4	0.4	0.6	0.9	1.3	0.8
PLS	0.2	0.2	0.7	0.3	0.5	1.8	2.9	1.9
POS	0.2	0.1	0.4	0.2	0.1	3.7	3.9	3.9
LnLnL	0.2	0.9	0.3	0.3	0.5	0.4	0.9	0.3
PoPL	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PoLnL	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PPLn	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
PLnS	0.1	0.0	0.3	0.1	0.0	0.7	0.0	0.6
LnLnS	0.1	0.0	0.4	0.2	0.0	0.4	0.0	0.3
LLS	0.1	0.0	1.0	0.5	0.8	3.3	1.9	2.6
SSLn	0.1	0.0	0.1	0.0	0.0	0.6	0.0	0.8
SSL	0.1	0.0	0.3	0.1	0.1	2.5	2.4	2.4
SSO	0.1	0.1	0.2	0.0	0.3	5.0	4.2	4.9
SSP	0.0	0.2	9.2	6.1	5.9	0.8	0.8	0.8
SSS	0.0	0.1	19.5	13.3	15.5	0.9	2.1	0.7
PPS	0.0	0.3	0.8	0.5	0.7	0.3	0.7	0.3
OOPo	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.1
PoLO	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1
PoOS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
PPP	0.0	1.0	0.0	0.0	0.1	0.0	0.0	0.0
LnLnLn	0.0	0.2	0.0	0.0	0.2	0.1	0.1	0.0
sum	99.9	99.8	99.8	99.6	100.1	100.1	100.0	99.8

[a] See footnotes in Table 1 for definitions and experimental conditions. [b] Area percent calculated using one set of response factors (normal method). [c] Area percent calculated using two sets of response factors, one set for the base oil, one set for the hardstock TAGs—PPS, SSP, SSS.

adjusted TAG area percent of the canola oil/soybean oil stearine blend gave a FA composition that was in better agreement than the unadjusted composition and which had an average error one-fourth the size of the error in the results by RP-HPLC-FID. Using this method, over-response or under-response by a few specific TAG species did not cause all TAG response factors to be affected. Instead, the three or four primary TAGs that accounted for a particular fatty acid were treated separately, whereas all of the other TAG response factors were handled normally.

Specifically, the canola oil/soybean oil tristearin blend was treated as follows: the areas of dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin were subtracted out; areas of dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin, which would normally be expected in the base oil, were calculated by using the average sizes of "stearic" and "palmitic" peaks in other chromatograms to predict the normal

Table 4. TAG Composition (Percent) of Genetically Modified High Stearic Acid Soybean Oil (SBO) Margarine Base Stocks by RP-HPLC with MS Detection and FID[a]

	SBO HS-1		A-6 first process		A-6 second process	
TAG	MS	FID	MS	FID	MS	FID
LLS	11.9	9.8	12.3	11.2	5.4	13.8
LLL	10.6	8.6	6.3	5.5	5.9	4.1
SSL	10.2	8.7	10.5	9.9	20.8	12.9
LLO	9.6	8.3	8.7	6.5	5.5	2.4
LLP	7.3	9.7	5.2	9.2	5.2	9.0
PLS	6.3	6.8	7.7	7.6	8.8	8.5
LOS	5.4	9.8	7.7	11.1	7.2	13.1
SSO	5.3	4.9	4.5	4.4	7.6	5.3
LLLn	4.0	2.9	3.4	2.3	3.2	2.2
PLO	4.0	7.8	4.1	7.4	2.5	6.4
OOO	3.1	3.2	1.2	1.7	1.2	0.9
LnLO	3.0	2.6	3.4	2.4	2.3	2.3
OOL	2.9	3.2	3.5	2.9	4.9	2.4
LnLS	2.8	0.0	3.5	0.0	4.7	0.0
POS	2.8	2.6	2.6	3.4	2.8	2.9
OOS	2.7	2.4	3.6	3.0	1.7	2.3
PPL	1.6	0.4	1.6	1.9	1.4	1.8
PLnL	1.4	1.9	1.6	2.0	1.6	1.7
LnOS	1.1	0.0	1.9	0.0	0.7	0.0
OOP	0.9	1.0	1.3	1.4	0.6	1.0
PLnO	0.6	0.4	0.8	0.8	0.7	0.3
LnLnL	0.5	0.4	0.5	0.5	0.5	0.4
OOLn	0.4	0.8	0.6	0.9	0.4	0.5
PPO	0.4	1.8	0.5	0.8	0.3	0.8
SSLn	0.3	0.0	1.2	0.0	1.7	0.0
LnLnS	0.2	0.0	0.3	0.0	0.5	0.0
SSS	0.2	0.3	0.3	0.6	0.6	0.4
LnLnO	0.2	0.2	0.2	0.3	0.2	0.3
PLnS	0.1	0.0	0.7	0.0	0.6	0.0
PPLn	0.1	0.1	0.2	0.0	0.2	0.2
SSP	0.1	0.6	0.2	0.5	0.4	2.0
LnLnP	0.1	0.1	0.1	0.1	0.2	0.1
PPP	0.0	0.1	0.0	0.4	0.0	0.4
PPS	0.0	1.1	0.1	1.3	0.1	1.7
sum	100.1	100.5	100.3	100.0	100.4	100.1

[a] See Table 1 for definitions and experimental conditions.

"stearic" and "palmitic" peak sizes in the dipalmitoyl, stearoylpalmitoyl, and distearoyl fragment ion chromatograms; these areas were calculated by using the average ratio of stearic acid and palmitic acid peaks to the sum of the peaks for oleic plus linoleic acid peaks in extracted ion chromatograms of palmitoyllinoleoyl (PL), palmitoyloleoyl (PO), dilinoleoyl (LL), linoleoyloleoyl (LO), and dioleoyl (OO) fragment ions. From these chromatograms, the average stearic/(linoleic + oleic) ratio was 0.04388, and the average palmitic/(linoleic + oleic) ratio was 0.18406. These ratios were multiplied by the (linoleic + oleic) areas in dipalmitoyl, palmitoylstearoyl, and distearoyl chromatograms to yield the fragment areas for palmitic and stearic that would normally be expected in the base oil due to the average sizes of palmitic and stearic peaks. These peaks gave calculated areas representing 0.09, 0.04, and 0.01% (very small amounts) for dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin, respectively, compared to 0.83, 5.18, and 6.06% in the raw data for the blend. The difference of the actual areas of dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin, minus the amount expected in the natural oil, gave the total area of these species attributed to the hard stock. The GC-FID FA composition of the base oil was found by subtracting 20 wt % from the total FA composition in the proportion of 10% palmitic and 90% stearic by weight (10.9% palmitic and 89.1% stearic mol %). The dipalmitoylstearoylglycerol, distearoylpalmi-

Table 5. FA Composition (Percent) of Corn Oil (CO) Margarine Base Stock Obtained Experimentally by GC-FID and Calculated from TAG Composition Listed for LC-MS and LC-FID in Table 1[a]

	CO					randomized CO					CO blend					interesterified CO blend				
FA	MS	AE[b]	FID	AE	GC	MS	AE	FID	AE	GC	MS[d]	AE	FID	AE	GC	MS	AE	FID	AE	GC
P	12.0	0.3	12.7	1.0	11.7	12.3	0.2	13.0	0.9	12.1	13.5	0.4	14.1	0.2	13.9	13.9	0.0	13.8	0.1	13.9
Ln	0.9	0.0	1.4	0.5	0.9	0.8	0.0	1.2	0.4	0.8	0.3	0.4	0.3	0.4	0.7	0.7	0.0	0.9	0.2	0.7
L	60.9	0.7	59.7	0.5	60.2	58.7	0.1	59.2	0.6	58.6	49.5	1.1	48.7	0.3	48.4	48.4	0.4	48.7	0.1	48.8
O	24.1	1.1	24.5	0.7	25.2	26.1	0.3	24.3	2.1	26.4	20.3	0.9	19.3	1.9	21.2	21.2	0.0	22.1	0.9	21.2
S	2.0	0.0	1.8	0.2	2.0	2.1	0.0	2.3	0.2	2.1	16.5	0.8	17.7	2.0	15.7	15.7	0.2	14.5	1.0	15.5
AAE[c]		0.4		0.6			0.1		0.8			0.7		1.0			0.1		0.5	

[a] See Materials and Methods and ref *26* for experimental conditions and data processing instructions for MS, FID, and GC FA and for preparation of oil samples. [b] Absolute error = absolute value of the error compared to GC for each FA. [c] Average absolute error = sum of the absolute error compared to GC for each FA divided by the number of FAs ($n = 5$). [d] Calculated FA composition from the TAG area percent calculated using two sets of response factors, one set for the base oil and one set for the hardstock TAGs—PPP, PPS, SSP, SSS.

Table 6. FA Composition (Percent) of Soybean Oil (SBO) Margarine Base Stock Obtained Experimentally by GC-FID and Calculated from TAG Composition Listed for LC-MS and LC-FID in Table 2[a]

	SBO					randomized SBOl					SBO blend					interesterified SBO blend				
FA	MS	AE	FID	AE	GC	MS	AE	FID	AE	GC	MS	AE	FID	AE	GC	MS	AE	FID	AE	GC
P	11.6	0.7	11.8	0.9	10.9	11.9	0.3	11.1	0.5	11.6	12.8	0.1	13.0	0.4	12.9	12.6	0.2	14.9	2.1	12.8
Ln	7.5	0.2	8.3	1.0	7.3	7.3	0.3	7.3	0.3	7.0	5.7	0.1	6.4	0.8	5.8	5.6	0.0	3.5	2.1	5.6
L	52.3	0.7	54.8	1.8	53.0	52.2	0.5	52.8	0.1	52.7	44.6	1.1	43.5	0.2	43.5	43.7	0.4	44.1	0.8	43.3
O	24.1	0.7	22.2	2.6	24.8	24.5	0.1	25.7	1.1	24.6	19.3	1.0	20.1	0.3	20.3	20.4	0.2	21.9	1.3	20.6
S	4.4	0.3	2.9	1.2	4.1	4.1	0.2	3.1	0.8	3.9	17.6	0.2	17.0	0.7	17.4	17.7	0.1	15.6	2.0	17.6
AAE		0.5		1.5			0.3		0.6			0.5		0.5			0.2		1.7	

[a] See footnotes in Table 2 for definitions and experimental conditions.

Table 7. FA Composition of Canola Oil (CNO) Margarine Base Stock Obtained Experimentally by GC-FID and Calculated from TAG Composition Listed for LC-MS and LC-FID in Table 3[a]

	CNO					CNO blend					interesterified blend				
FA	MS	AE	FID	AE	GC	MS2[b]	AE	FID	AE	GC	MS	AE	FID	AE	GC
P	4.9	0.3	7.0	1.8	5.2	8.3	0.1	9.1	0.9	8.2	7.5	0.0	11.5	4.0	7.5
Ln	14.1	0.3	13.8	0.6	14.4	6.3	0.4	8.2	1.5	6.7	7.2	0.0	5.2	2.0	7.2
L	19.7	0.2	17.8	2.1	19.9	20.4	0.3	18.5	2.2	20.7	21.2	0.2	23.7	2.3	21.4
O	59.6	0.8	60.1	1.3	58.8	45.6	1.0	42.9	1.7	44.6	44.5	0.0	41.4	3.1	44.5
P	1.7	0.0	1.4	0.3	1.7	19.4	0.3	21.3	1.6	19.7	19.3	0.1	18.2	1.0	19.2
AE		0.3		1.2			0.4		1.6			0.1		2.5	

[a] See Table 2 for definitions and experimental conditions. [a] Calculated FA composition from the TAG area percent calculated using two sets of response factors, one set for the base oil and one set for the hardstock TAGs—PPS, SSP, SSS.

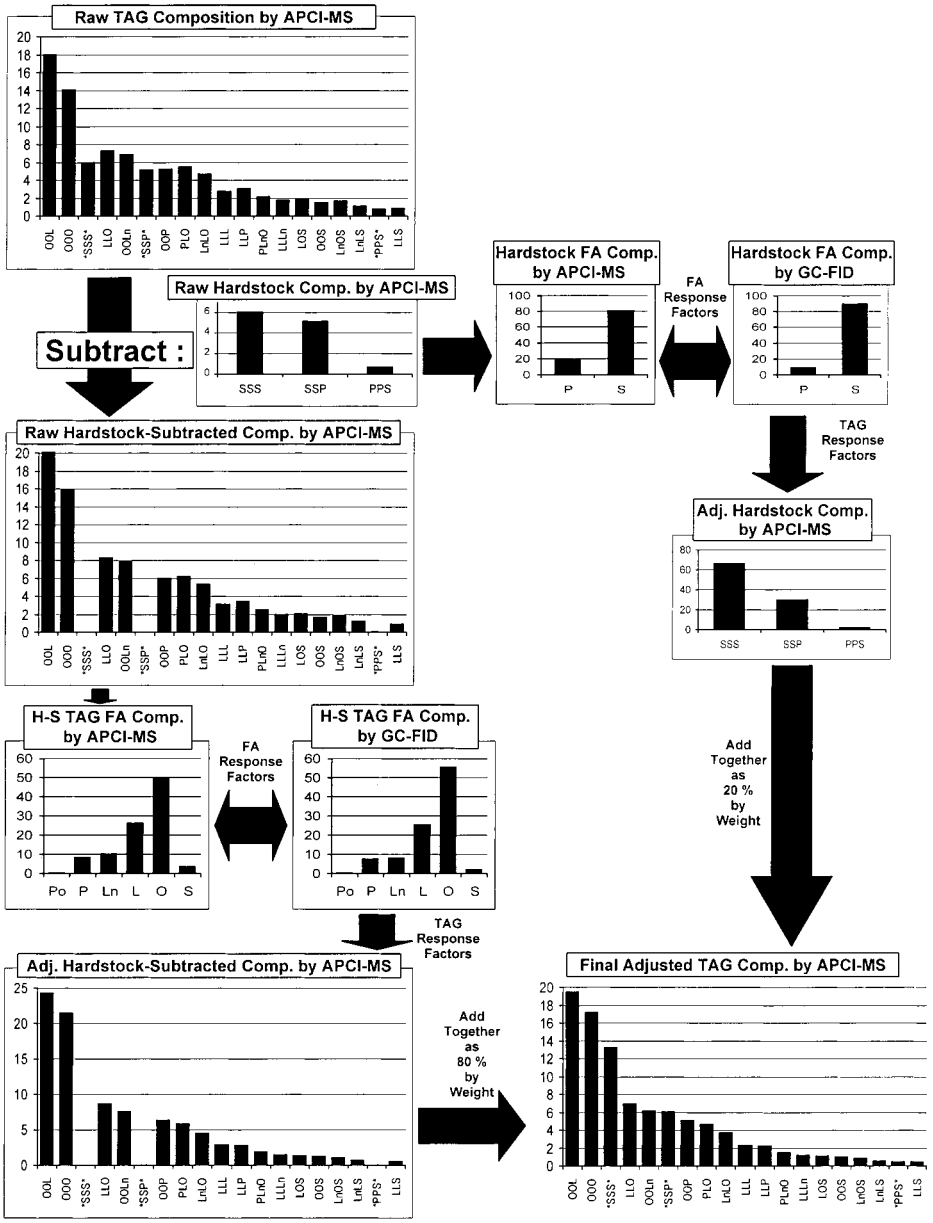
Table 8. FA Composition of Genetically Modified Soybean Oil (SBO) Margarine Base Stock Obtained Experimentally by GC-FID and Calculated from TAG Composition Listed for LC-MS and LC-FID in Table 4[a]

	SBO HS-1					A-6 first processing					A-6 second processing				
FA	MS	AE	FID	AE	GC	MS	AE	FID	AE	GC	MS	AE	FID	AE	GC
P	9.2	0.2	13.2	4.2	9.0	9.6	0.4	13.0	3.8	9.2	9.1	0.4	14.1	5.4	8.7
Ln	5.3	0.0	3.3	2.0	5.3	6.5	0.0	3.5	3.0	6.5	6.2	0.1	3.0	3.1	6.1
L	45.1	0.4	43.7	1.0	44.7	40.6	0.7	41.2	0.1	41.3	36.9	0.6	38.1	0.6	37.5
O	18.4	1.2	18.3	1.3	19.6	18.6	0.7	21.0	1.7	19.3	16.1	0.8	16.0	0.9	16.9
S	22.0	0.5	21.5	0.0	21.5	24.6	0.9	21.3	2.4	23.7	31.8	0.9	28.8	2.1	30.9
AE		0.5		1.7			0.5		2.2			0.6		2.4	

[a] See Table 2 for definitions and experimental conditions.

toylglycerol, and tristearin peaks initially gave an FA area percent of 18.6% palmitic and 81.45% stearic. Response factors were calculated and applied so that the adjusted composition of the hard stock TAGs had an FA area percent of 11.8% palmitic and 88.2% stearic, compared to the molar percentages by GC-FID, above. The response factors were 1.0000 and 1.8572 for palmitic and stearic in the canola blend hard stock TAGs, respectively. The adjusted "hard stock-subtracted TAG composition" was converted to a weight percent, and the adjusted hard stock TAG composition was added back in so that it represented 20% of the total blend weight. The area percents are given in Table 3 for the blend treated using the normal method of calculating response factors (column MS1) and the area percents after treatment with the modified procedure using two sets of response factors (column MS2). The percentages of all stearic-containing TAGs is larger in the data obtained using the unmodified method, because using that method only one set of response factors was applied to all stearic-containing species. Table 7 shows comparison of the FA composition calculated from the TAG composition with that obtained by GC-FID. The unadjusted ("raw") area TAG percent gave an average absolute error of 2.3% per fatty acid, the area percent adjusted using the normal method for response factor calculation gave an average of 4.3% absolute error per fatty acid, and the area percent adjusted using the "blend modification" of the previous procedure produced only 0.41% average absolute error and the RP-HPLC-FID data produced 1.6% average absolute error. Thus, the blend modification of the previously developed method dem-

Scheme 2



onstrated the lowest average error in the FA composition.

Corn and Soybean Oil Blends. The corn and soybean oil blends in Tables 1 and 2 were treated the same as the canola/soybean oil stearine blend, except that four instead of three TAGs (tripalmitin, dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin) were treated as the primary hard stock TAGs and so had the second set of response factors applied to them. The corn and soybean oil blends utilized cottonseed oil tristearin flakes. The FA composition of these has previously been reported (*13*) Although the hard stock contained small amounts of other TAGs, only the primary four TAGs mentioned above were present in such quantities that they required normalization using separated response factors. The FA composition of the four TAG species is given by the relative amounts of palmitic and stearic reported earlier for this hard stock, recalculated to equal 100%. The corn oil blend gave a response factor of 1.3031 for stearic in the hardstock, and the soybean oil blend gave a response factor of 1.3574 for stearic, with the factor for palmitic set to 1.0000. Before application of the modified method for response factor calculation, the "raw" corn oil blend TAG composition resulted in an FA composition that had an average absolute error of 7.3%, but the FA composition obtained after application of the modified response factor calculation procedure brought the average absolute error to 0.6%. Similarly, the unadjusted soybean oil blend TAG composition resulted in an FA composition that had an average absolute error of 3.1%, but the blend modification of the method for response factor calculation gave an average absolute error of 0.5%.

Table 2 lists the TAG compositions of normal and randomized soybean oil and of the soybean oil−cottonseed oil stearine blend and interesterified blend. Figure 1A shows a chromatogram of the blend of soybean oil with cottonseed oil saturates obtained by RP-HPLC-FID. The three large peaks in Figure 1 eluted at long retention times represent the major components of the cottonseed oil saturates, determined by RP-HPLC-FID to be composed of tripalmitin (0.7%), dipalmitoylstearoylglycerol (10.1%), distearoylpalmitoylglycerol (35.1%), and tristearin (54.1%). Figure 2A shows

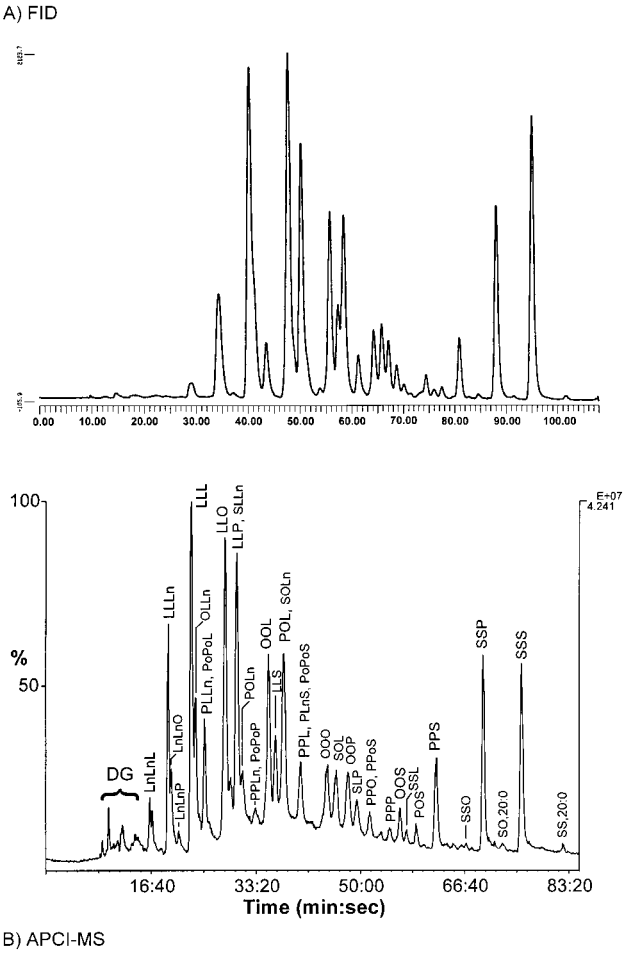


Figure 1. (A) LC-FID and (B) LC-APCI-MS chromatograms of soybean oil−cottonseed stearine blend (80:20 wt %). Peak labels identify individual TAG molecular species. P, Ln, L, O, and S represent palmitic, linolenic, linoleic, oleic, and stearic acids of the TAG, respectively. See Materials and Methods for analytical conditions.

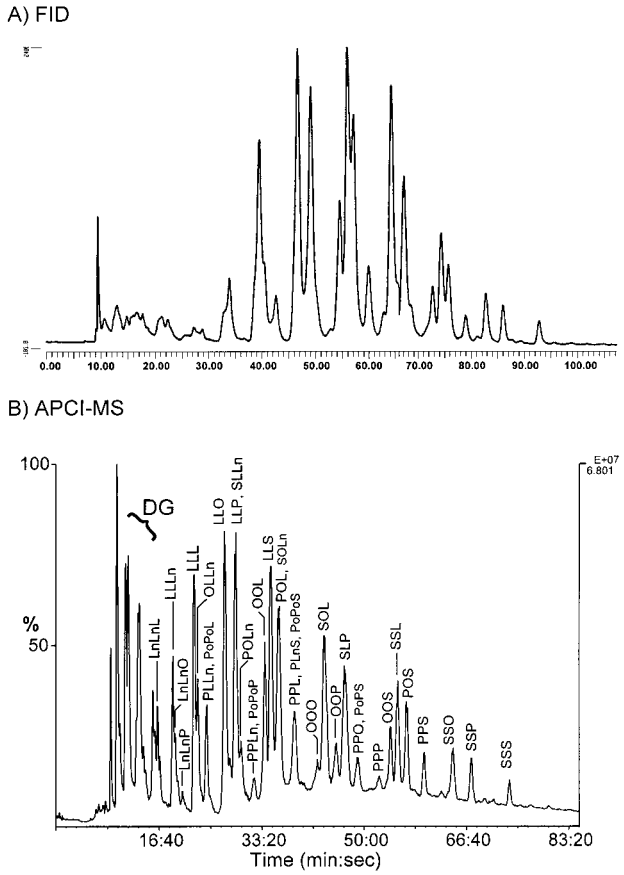


Figure 2. (A) LC-FID and (B) LC-APCI-MS chromatograms of interesterified soybean oil−cottonseed stearine blend (80:20 wt %). See Figure 1 for abbreviations and experimental conditions.

a chromatogram of the interesterified blend obtained by RP-HPLC-FID. The difference in the composition of individual saturate-containing molecular species is clearly seen by comparison of Figurs 1 and 2. The diminished size of the trisaturated TAG in Figure 2 compared to Figure 1 and the increased sizes of stearic acid-containing TAGs (such as distearoyllinoleoylglycerol and stearoyloleoyllinoleoylglycerol) show the effect of interesterification. The effect of interesterification is shown more clearly in the extracted ion chromatograms shown in Figures 3 and 4 for the soybean oil/cottonseed oil stearine blend and interesterified blend, respectively. In Figure 3, each extracted ion chromatogram contained only a relatively small peak arising from stearic acid, except the extracted ion chromatograms of hard stock: the distearoyl chromatogram contained mostly palmitoyl and distearoyl peaks, and the dipalmitoyl chromatogram contained a large stearoyl peak. Conversely, Figure 4 shows a large stearoyl acid peak in each chromatogram and a dramatic change in the peaks in the "distearoyl" extracted ion chromatogram, because the stearic acid was redistributed among all possible molecular species by interesterification.

Table 3 lists the TAG compositions of normal and randomized canola oil and of the canola oil−soybean oil stearine hard stock blend and interesterified blend. Also, the TAG compositions determined by RP-HPLC-FID of high-stearic oils from genetically modified soybean varieties HS-1 and A-6 are given in Table 4.

The liquid vegetable oil blends and interesterified products are presented as examples of products that can be developed from readily available components to make margarine base stocks. The high-stearic oils are presented as examples of intrinsically high saturated FA oils, which are now available from genetically modified oilseed varieties. These high-stearic oils may contain enough solid TAGs to be suitable, perhaps with randomization but without blending or interesterification, for margarine base stocks. Because randomized or interesterified mixtures should have compositions given by the statistical distribution of their FA compositions, the difference of the TAG composition determined using RP-HPLC from the TAG composition predicted from the statistical distribution of the fatty acid among all possible TAG species gave a value for average absolute error in the TAG composition, just as was calculated for the FA composition. The average absolute error of the interesterified corn oil was 0.45% for the adjusted APCI area percent versus 0.57% average absolute error for the RP-HPLC-FID TAG composition. The average absolute error of the corn oil interesterified blend was 0.27% for the adjusted APCI area percent versus 0.36% average absolute error for the RP-HPLC-FID TAG composition. The average absolute error of the interesterified soybean oil area percent obtained by APCI-MS was 0.23% compared to 0.35% for the RP-HPLC-FID TAG composition. For the soybean oil interesterified blend area percent obtained by APCI-MS, the average absolute error was 0.23% compared to 0.64% for the RP-

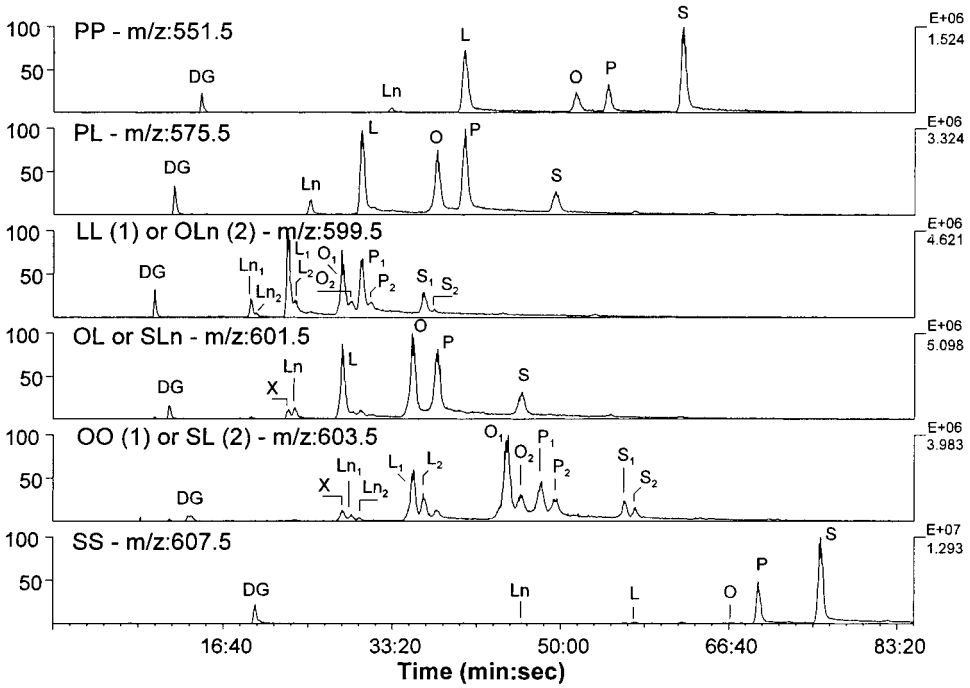


Figure 3. Extracted ion chromatograms of $[M - RCOO]^+$ *m/z* from soybean oil−cottonseed stearine blend. $[M - RCOO]^+$ identity and *m/z* are given in the upper left of each chromatogram. The peaks are labeled with the identity of the fatty acyl chain (RCOO) which, combined with the $[M - RCOO]^+$ fragment, makes each of the TAG molecular species. See Figure 1 for abbreviations and experimental conditions.

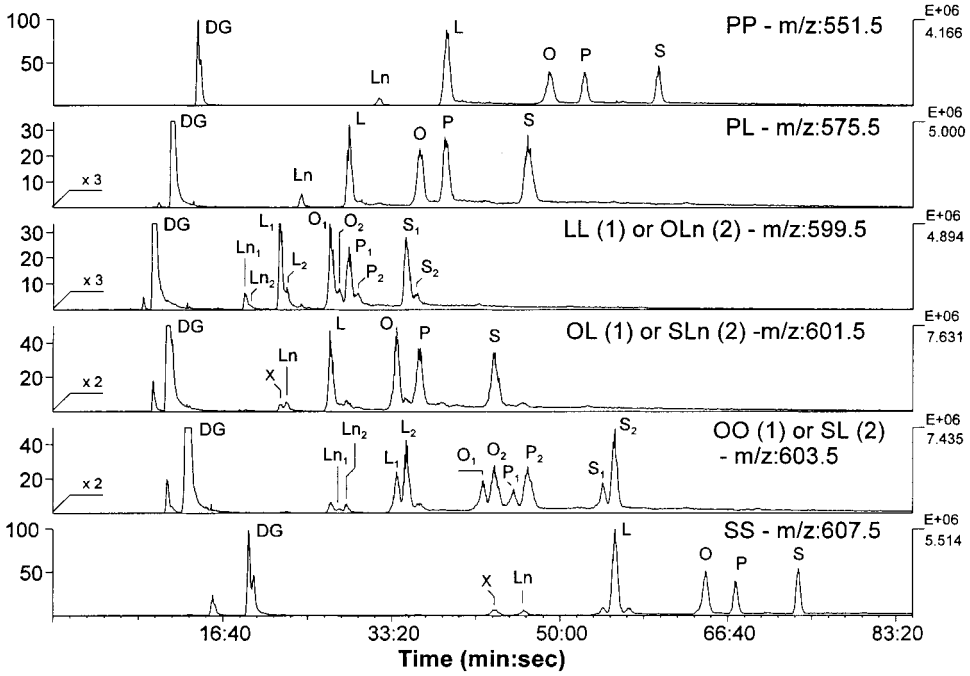


Figure 4. Extracted ion chromatograms of $[M - RCOO]^+$ *m/z* from interesterified soybean oil−cottonseed stearine blend. $[M - RCOO]^+$ identity and *m/z* are given in the upper left of each chromatogram. The peaks are labeled with the identity of the fatty acyl chain (RCOO) which, combined with the $[M - RCOO]^+$ fragment, makes each of the TAG molecular species. See Figure 1 for abbreviations and experimental conditions.

HPLC-FID TAG composition. Thus, in every case, the adjusted APCI-MS composition was closer to the known composition than that given by RP-HPLC-FID. This comparison to the statistically predicted composition may not be made for normal oil or non-interesterified blends because the fatty acids in these oils are not expected to be completely randomly distributed.

Identification and Quantitation of Margarine Base Stock TAGs. Because base stock physical properties depend on the TAG composition, it is important to have good analytical procedures to analyze the TAG compositions of base stocks. Also, for quality control, a TAG analysis should be performed on the vegetable oils to be used in base stock formulation. For example, the TAG composition of the A-6 soybean oil (Table 4) varied depending on the lot from which the seed samples were taken. The adequacy of the TAG composition analysis was determined by calculating the FA compositions from the TAG compositions obtained by RP-HPLC-FID or RP-HPLC/APCI-MS and comparing them with the

FA compositions obtained experimentally from calibrated GC-FID analysis of the methyl esters of the same oil sample. The FA analysis by GC-FID is considered to be very reliable, because each fatty acid is referenced to a standard mixture of fatty acid of known composition by weight.

The utilization of extracted ion chromatograms allowed identification and quantitation by APCI-MS of chromatographically overlapped TAG pairs such as dilinoleoylpalmitoylglycerol and stearoyllinoleoyllinolenoylglycerol, palmitoyloleoyllinoleoylglycerol and stearoyloleoyllinolenoylglycerol, and dipalmitoyllinoleoylglycerol and palmitoyllinolenoylstearoylglycerol. Such identification and quantitation were not possible using the RP-HPLC-FID system. Examples of extracted ion chromatograms for the soybean oil−cottonseed oil stearine blend and interesterified blend are presented in Figures 3 and 4. These extracted ion chromatograms represent the $[M - RCOO]^+$ fragment ions of the TAG formed in the APCI source. The extracted ion chromatograms are invaluable for TAG identification, and the areas under the peaks in the extracted ion chromatograms were combined to provide total areas attributable to each of the TAG species. However, due to differences in response arising from different fragmentation patterns of unsaturated versus saturated species, integration of the peak areas did not give accurate TAG quantitation without response factors.

The smaller error associated with the APCI-MS data resulted largely from the ability of the MS data to differentiate chromatographically overlapped TAG species. The RP-HPLC-FID peaks were often attributed to one TAG when they actually contained several species. This became especially important for species present in low amounts. To allow direct comparison of the TAG species identified by RP-HPLC-FID, the quantitation of numerous palmitoleic acid-containing species identified by APCI-MS was not included in this treatment. However, many species were conclusively identified and readily quantified in amounts as low as 0.01%.

Knowledge of accurate TAG composition of margarine base stocks is important for understanding what TAG composition is responsible for certain important physical properties of margarines. For example, lubricity, or mouthfeel of a food product (that is, how well the food product melts in the mouth to give a pleasant, cooling effect), is an important physical property related to TAG species and quantity (*10*). TAGs that are disaturated or trisaturated are most important for this effect. Examination of the RP-HPLC/APCI-MS and RP-HPLC-FID TAG composition data in Tables 1−4 can be made for base stock products that have high contents of di- and trisaturated TAGs. Genetically modified soybean varieties HS-1 and A-6 had high concentrations of disaturated TAGs such as distearoyllinoleoylglycerol and distearoyloleoylglycerol. Normal corn, soybean, and canola oil blends with cottonseed oil stearine and soybean oil stearine hard stocks had high concentrations of trisaturated TAGs such as dipalmitoylstearoylglycerol, distearoylpalmitoylglycerol, and tristearin. Interesterified blends had higher concentrations of di- and trisaturated TAGs than the starting liquid oils. Selection of base stock products with these TAG types may assist development of margarines with good lubricity or mouthfeel. Similarly, spreadability, tub stability, and other important characteristics depend on the presence of specific TAG species. The effort to link texture and flavor characteristics with structural properties is ongoing, and the ability to produce a good estimate of a product's TAG composition will be central to gaining a better understanding of the relationshiop between TAG form and function.

It has been demonstrated that the analytical tools are now available to provide necessary information for more thorough characterization of oil products than ever before. In the case of natural oils and interesterified or randomized oils, APCI-MS was shown to provide quantitation which was better than that given by RP-HPLC-FID, a commonly used method in use for routine TAG analysis. Only in the case of non-interesterified blends did the APCI-MS method require modification. Thus, some understanding of the nature of the sample being analyzed can lead to the best choice of the method used for analysis. Budgetary constraints also play an important role in the choice of analytical methodology. It was shown that the RP-HPLC-FID method provided results which were generally similar to those obtained by RP-HPLC/APCI-MS and so may be sufficient as an economical alternative to the more accurate, but much more expensive, mass spectrometric method.

ABBREVIATIONS USED

RP-HPLC, reverse-phase high-performance liquid chromatography; APCI-MS, atmospheric pressure chemical ionization mass spectrometry; MS, mass spectrometry; FA, fatty acid; TAG, triacylglycerol; FID, flame ionization detection.

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