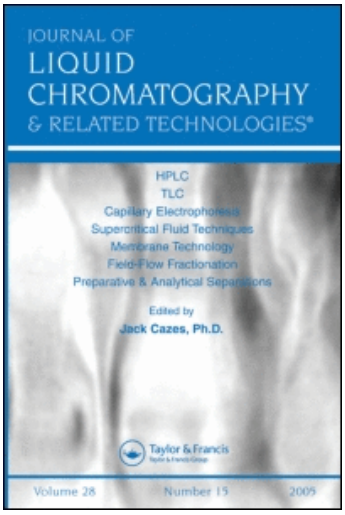

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analyses of Vegetable Oil Triglyceride Molecular Species by Reversed Phase High Performance Liquid Chromatography

M. A. M. Zeitoun[a]; W. E. Neff[b]; E. Selke[b]; T. L. Mounts[b]
[a] Department of Food Science, Alexandria University Faculty of Agriculture, Alexandria, Egypt [b] Department of Agriculture, Food Quality and Safety Research National Center for Agricultural Utilization Research United States, Peoria, Illinois

To cite this Article Zeitoun, M. A. M. , Neff, W. E. , Selke, E. and Mounts, T. L.(1991) 'Analyses of Vegetable Oil Triglyceride Molecular Species by Reversed Phase High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 14: 14, 2685 — 2698
To link to this Article: DOI: 10.1080/01483919108049348
URL: http://dx.doi.org/10.1080/01483919108049348

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(14), 2685-2698 (1991)

ANALYSES OF VEGETABLE OIL TRIGLYCERIDE MOLECULAR SPECIES BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

M. A. M. ZEITOUN[1], W. E. NEFF[2]*,
E. SELKE[2], AND T. L. MOUNTS[2]
[1]*Visiting Scientist from Alexandria University*
Faculty of Agriculture
(Saba Basha) Department of Food Science
Alexandria, Egypt
[2]*Food Quality and Safety Research*
National Center for Agricultural Utilization Research
United States Department of Agriculture
1815 North University Street
Peoria, Illinois 61604

ABSTRACT

Triglyceride molecular species (TGMS) of 10 vegetable oils (olive, soybean, sunflower, corn, cottonseed, pumpkin seed, peanut, safflower, canola and palm oil) were separated and analyzed quantitatively by gradient, reversed phase high performance liquid chromatography with a flame ionization detector (FID). Identification of TGMS was made by comparison of experimental and calculated theoretical carbon numbers (TCN). The relationship between elution time and calculated TCN of each TGMS was linear. The FID response (area percent) was determined to be linear or proportional to weight percent. Nine of the oils showed significant differences between observed TGMS composition and

*Author to whom correspondence should be addressed.

composition expected or calculated from an assumption of random fatty acid distribution. Results indicate preferential or genetically controlled distribution of fatty acids in TGMS of these oils and a random distribution for sunflower oil. TGMS composition was determined for a 1:1 blend of soybean oil with fully hydrogenated soybean oil before and after interesterification.

INTRODUCTION

Oxidative stability of seed oils is related to triglyceride composition (TGC) (1,2). Further, oxidation of oils can produce varying quantities of hydroperoxides and secondary oxidation products depending on the triglyceride molecular species (TGMS) composition (1). These oxidation products have flavor, odor and biological implications (3). Also, melting behavior and polymorphism of fats depend mainly on TGC (4). TGC is a reliable method to identify a fat and to determine adulteration. (5). Studies of genetic control of fatty acid distribution in vegetable oil triglycerides depend upon knowledge of TGMS composition. Further, studies of changes in oil physical structure and functionality by chemical (example, partial hydrogenation) or enzyme directed reactions (example, lipase directed interesterification) require analysis to evaluate changes in TGMS (6).

Christie reviewed recent progress on the separation of TGMS by reversed-phase high-performance liquid chromatography (RP-HPLC) (7). He concludes that the present best analytical system utilizes a detector based on the transport flame ionization principle (FID) which allows a wide use of solvents and gives quantitative response. Nurmela et al. reported that other HPLC detectors for triglyceride analysis, i.e., refractive index, ultraviolet, infrared, mass or light scattering, have low sensitivity, gradient incompatibility, and variable or non-linear response (5). Christie states that the best mobile phase system for resolution of TGMS of different polarities, as found in vegetable oils, is a gradient mixture of acetonitrile and methylene chloride (7).

He also reported that the retention times or elution volume of TGMS are best described by theoretical carbon number (TCN). TCN considers other elution effects in addition to total carbon and double bond numbers associated with the previously used equivalent carbon number. These effects concern the different impact that the saturated and mono-, di- and tri-unsaturated (cis and trans) fatty acid moieities have on the elution of TGMS (7). Nurmela et al. used gradient mobile phase elution on a reversed phase column (GRP-HPLC) with FID for analysis of butterfat (5). RP-HPLC with linear and isocratic mobile phases and FID has been used for the analysis of TGMS in cocoa butter, soybean and olive oil (8-9).

Application of quantitative GRP-HPLC with FID to the TGMS analysis of 10 commercially important vegetable oils and a study of the products of interesterification reaction of a blend of soybean oil with hydrogenated soybean oil is reported here.

EXPERIMENTAL

Materials

The following purified simple triglycerides (TGS) were obtained from NuChek Prep (Elysian, MN): trilaurin, tripalmitin, tristearin, trilinolein, and trilinolenin. Vegetable oils: olive, soybean, sunflower, corn, cottonseed, pumpkin seed, peanut, safflower, canola and palm oil were obtained from either local market or industrial sources as finished edible oils. An interesterified oil was prepared by the sodium methoxide catalyzed interesterification of a 1:1 (wt/wt) blend of soybean oil and fully hydrogenated soybean oil. Solid phase extraction (SE) columns (6.5 ml, loaded with 2 g silica) were purchased from Baxter Health Care (Muskegen, MI). HPLC grade acetonitrile and methylene chloride were filtered through a 45 micron disc and degassed before use.

Methods

Pure TGS were prepared from vegetable oils by SE chromatography, as described previously (1). The oil stripping involved SE chromatography with a hexane-diethyl ether/hexane-

methanol gradient of 1.2 g oil mixed with 0.485 g activated carbon. Purity of the TG was evaluated by Thin Layer Chromatography with diethyl ether:hexane (20:80, v/v) and visualization of resolved components by iodine and by sulfuric/chromic acid charring.

TGS (0.5 mg in 5-10 ul hexane) were resolved by RP-HPLC (1,8) with linear gradient elution program (0.8 ml/min acetonitrile: methylene chloride (60:40 to 30:70, v/v) over 120 min), followed by column clean-up with 100% methylene chloride. Two C-18 (5 u) 0.49 x 50 cm Zorbax columns (Dupont Inst., Wilmington, DE) were used in series. The flame ionization detector was a Tracor Model 945 HPLC detector (Austin, TX) (5), block temperature:180 C; detector and cleaning flame gas flow rates: 140 and 600 ml/min hydrogen respectively and 300 ml/min oxygen. The noise filter was set high and base line correction was used. FID output was monitored by a real-time computer programmed to calculate peak area from solute responses. (10).

Fatty acid methyl esters (FAME) were prepared by potassium methoxide catalyzed transmethylation of TGS (1). FAME were analyzed by GC using a direct injection column (6 ft x 0.125 in) packed with 10% SP 2330 on chromosorb W(AW) 100/120 mesh (Supelco, Inc., Bellefonte, PA). The column was operated isothermally at 160 C with a helium flow rate 20 ml/min. The injector and FID temperatures were set at 200 C. Sample size was 1 μl of 0.5% solute in diethyl ether. Chromatogram peak integration was by computer as described above (10). The GC area percent was calibrated against weight percent of NuChek Prep FAME mixture 15A, and indicated that no response factors were required.

Calculation of vegetable oil TGMS composition was performed according to the random distribution of fatty acids as discussed by Merritt et al. (9), Vander Wal et al. (11) and by Stirton (12).

RESULTS AND DISCUSSION

The TGMS in each vegetable oil were identified by matching their calculated and experimental TCN values (13) relative to

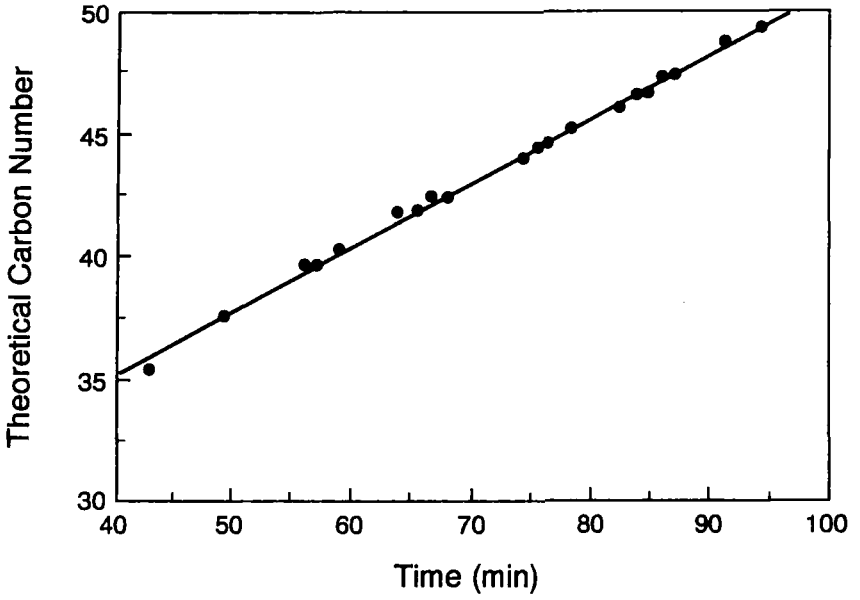

FIGURE 1. Relationship between theoretical carbon number and HPLC elution time for soybean oil triglyceride molecular species.

elution times and from the elution order of known TGMS in reference mixtures. The relationship of retention time and TCNwas found to be linear for GRP-HPLC as shown for soybean oil in Figure 1.

An example of a GRP-HPLC chromatogram is given in Figure 2 for TGMS of a 1.1 blend of soybean oil and hydrogenated soybean oil before (A) and after (B) intersterification.

Thus in the GRP-HPLC system, the retention times are dependent on the sum of fatty acid chain lengths and number, geometry and positions of double bonds as found for isocratic systems (7). Fatty acid designation in TGMS in Figure 2 or in the Tables does not indicate positional isomer resolution, i.e. LLO likely includes LOL.

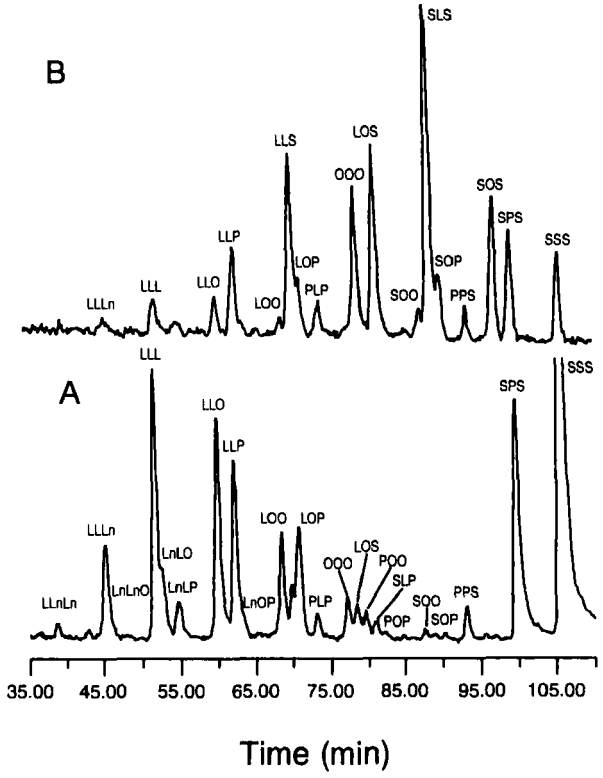

FIGURE 2. Gradient reversed phase HPLC with flame ionization detection. Chromatograms of blend (1:1) of soybean oil and fully hydrogenated soybean before (A) and after interesterification (B). Ln, L, O, P, S are linolenic, linoleic, oleic, palmitic and stearic fatty acids of the triglyceride molecular species detected. HPLC conditions are in methods section in the text.

Results of GRP-HPLC of four standard mixtures of pure triglycerides of known weight are presented in Table 1. The TGS contents were each varied over a wide range in the four mixtures to be representative of their occurrence in different vegetable oils. Analyses were performed in triplicate with a standard deviation (14) of 0.7% or less.

TABLE 1

Gradient Reverse Phase HPLC[a] of Synthetic Triglyceride Mixtures[b]

Synthetic Triglyceride Mixture		Area Percent (HPLC) and Weight Percent Composition				
		TriLn	TriL	TriO	TriP	TriS
1.	HPLC[c]	17.6	17.9	20.5	22.9	21.1
	Wt. Percent[b]	17.9	19.3	21.5	21.8	19.5
	CV (%)[d]	1.2	5.4	3.4	3.5	5.4
2.	HPLC[c]	20.7	28.6	33.3	7.0	10.4
	Wt. Percent[b]	20.0	30.5	32.5	6.5	10.0
	CV (%)[d]	2.5	4.5	1.7	5.2	2.8
3.	HPLC[c]	13.8	56.7	18.1	9.2	2.2
	Wt. Percent[b]	13.0	55.6	19.5	9.8	2.1
	CV (%)[d]	4.2	1.8	5.3	4.5	3.3
4.	HPLC[c]	5.3	36.6	30.3	16.7	11.1
	Wt. Percent[b]	5.2	36.3	31.0	17.0	10.5
	CV (%)[d]	1.4	0.6	1.6	1.3	3.9

[a] See Methods section in text for HPLC conditions.

[b] Mixture of Trilinolenin, Trilinolein, Triolein, Tripalmitin and Tristearin, prepared by weight.

[c] HPLC peak area percent. S.D. $\pm$ 0.1 - 0.7. Analyses performed in triplicate.

[d] CV = Coefficient of variation (13).

TABLE 2

Vegetable Oils: Calculated and Experimental Composition

Molecular Species[a]	Canola		Corn		Cottonseed		Olive		Palm	
	Calc.[b]	Obs.[c]	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
LnLL			1.2	2.1						
LnLnO	1.2	1.3								
LLL			21.4	25.2	14.6	15.6				
LnLO	6.1	5.4								
LLO	8.1	7.0	28.0	26.1	14.4	11.8	3.5	1.3	1.2	1.0
LnOO	9.6	11.2					1.2	0.8		
LLP			12.2	15.2	21.5	25.4	0.8	0.3	1.2	1.5
LnOP	1.3	1.4								
LOO	25.1	23.5	12.2	10.4	3.8	4.7	17.7	10.0	5.3	1.2
LIS			1.8	1.8	2.0	1.8				
LOP	3.5	4.1	10.6	9.9	14.1	12.8	8.1	4.0	10.5	8.3
LPP	0.1	1.2	2.3	2.0	10.6	15.8	0.9	0.3	5.2	6.2
OOO	26.1	34.6	1.8	3.7	0.5	1.4	29.2	49.7	7.9	3.8
LOS			1.6	1.5	1.3	1.0				
POO	5.4	5.3	2.3	2.2	2.3	2.4	20.1	22.0	23.5	30.4
LPS			2.0	1.7						
PPO	0.4	1.3			3.5	4.4	4.6	2.4	23.4	36.2
PPP					1.7	0.4			7.7	3.1
SOO	2.3	2.2			0.2	0.2	3.4	5.3	2.2	1.8
SOP					0.6	0.3	1.6	0.8	4.4	5.3
PPS							0.2	0.4	2.2	0.8

[a] Ln, L, O, P, S are linolenic, linoleic, oleic, palmitic and stearic acids respectively. Some molecular species possible from random fatty acid distribution: LnLnP, LnLnS, LnLS, LnPP, LnPS, LnSS were not found by HPLC.

[b] Composition calculated from fatty acid data (Table 4) (9-10, 14). Expected TG not listed or for which no value is given are < 1%.

[c] Composition peak area percent. HPLC conditions in methods analysis in triplicate. TG not listed or for which no value is given are < 1%.

TABLE 3

Vegetable Oils: Calculated and Experimental Composition

Molecular Species[a]	Soybean		Peanut		Pumpkin		Safflower		Sunflower	
	Calc.[b]	Obs.[c]	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
LnLL	4.8	5.4							0.7	0.3
LLL	12.7	15.2	2.9	1.7	16.5	20.7	42.9	49.7	34.6	35.8
LnLO	5.4	3.6								
LnLP	2.2	2.4								
LLO	21.1	16.8	14.5	12.7	23.0	19.9	25.4	18.1	26.3	26.6
LnOO	1.5	1.0								
LLP	8.5	13.5	3.8	3.0	11.8	16.5	11.6	14.8	10.3	10.7
LnOP	1.2	0.4								
LOO	11.7	10.5	24.4	19.9	10.7	9.2	5.0	3.7	6.6	6.0
LLS	3.0	3.0			5.0	5.5	4.1	4.0	6.7	7.4
LOP	9.7	10.1	9.8	11.9	11.0	11.9	4.6	3.7	5.2	5.4
LPP	2.0	2.1	1.0	1.8	2.9	3.5	1.0	0.4	1.0	0.8
LO 20:1			1.1	1.1						
OOO	2.2	4.8	12.7	17.9	1.7	3.1	0.3	3.1	0.8	1.5
LOS	3.4	3.2	2.5	1.4	4.7	4.0	1.6	1.2	3.4	2.7
POO	2.7	3.5	7.9	10.0	2.6	2.0	0.5	1.0	0.7	0.7
LPS	1.4	1.3	0.5	2.7	2.4	2.8	0.7	0.3	1.3	0.9
POP	1.1	0.7	1.7	3.0					0.3	0.7
OL 20:0			1.3	0.7						
SOO	0.9	1.3	2.6	2.9	1.1	0.7			0.4	0.2
OL 24:0			2.8	2.8						

NOTE: Footnotes same as in Table 2.

The data show that HPLC area percent is within a Coefficient of Variation (14) of 5% or less compared to the known weight percent of simple triglycerides. Thus, the FID response requires no response factors for quantitative analysis. This finding is in agreement with the work of Nurmela et al. (5) and Phillips et al. (8).

Quantitative analysis of TG from ten vegetable oils by GRP-HPLC was compared to the calculated composition based on random fatty acid distribution and the results are shown in Tables 2 and 3.

TABLE 4

Fatty Acid Composition of Selected Vegetable Oils

	Fatty Acid GLC (%) [a]											
Oil	12:0	14:0	16:0	18:0	20:0	22:0	24:0	16:1	18:1	18:2	18:3	20:1
Canola			4.3	1.8					63:0	20.7	8.9	1.5
Corn			11.3	1.7					26.1	59.8	1.1	
Cottonseed		0.7	25.9	2.4				0.7	17.3	52.6	0.4	
Olive			15.2	2.6				1.6	66.4	13.3	0.9	
Palm		0.9	42.6	4.0					42.9	9.6		
Peanut		0.0	10.5	2.7	1.4	2.0	1.0		50.2	31.0		1.2
Pumpkin			13.1	5.6				0.2	25.5	54.8	0.8	
Safflower			6.8	2.4					14.9	75.4	0.5	
Soybean			11.5	4.0					27.9	50.2	6.4	
Sunflower			7.0	4.5					17.8	70.2	0.5	

[a] See Methods section for GLC procedures.

The calculated composition is derived from the fatty acid composition of the vegetable oils determined by GC of FAME, as presented in Table 4.

Variation determined between the HPLC area percent and the calculated composition, divided by the calculated composition, defined as E, was significant for most of the major TGMS (>10%) of 9 of the 10 vegetable oils. Maximum E ranged from 26 for LLL of pumpkin to 70 for OOO of olive oils. These data indicate that the TGMS of the nine oils (soybean, peanut, pumpkin, safflower, canola, corn, cottonseed, olive and palm oils) are not in agreement with the theory of random fatty acid distribution (12,15).

These oils may exhibit a preferentially or genetically controlled distribution of fatty acids (16,17). However for sunflower oil, the variation was less than an E of 4 for TGMS

TABLE 5

Composition by Gradient Reverse Phase HPLC[a] of Soybean and Hydrogenated Soybean Oil Blend Before and After Interesterification

Molecular Species[b]	HPLC Area Percent Interesterification: SBO + Hydrogenated SBO Before[c]	After[d]
LLLn	4.4	1.4
LLL	10.6	2.0
LnLO	2.9	0.8
LnLP	1.8	1.2
LLO	10.1	2.6
LLP	8.2	5.4
LOO	4.4	0.9
LLS	1.8	11.1
LOP	4.8	3.3
PLP	1.0	2.1
OOO	1.7	9.6
LOS	1.4	12.6
POO	1.1	0.0
SOO	0.4	1.6
SLS	0.0	20.7
SOP	0.3	3.9
PPS	1.3	1.6
SOS	0.0	7.9
SPS	13.3	5.9
SSS	28.4	5.0

a See Footnote a, Table 1.

b Ln, L, O, P, S are linolenic, linoleic, oleic, palmitic and stearic acids respectively.

c Molecular species of a 1:1 (wt/wt) blend of soybean and hydrogenated soybean oils.

d Molecular species of a 1:1 (wt/wt) blend of soybean and hydrogenated soybean oils after interesterification with sodium methoxide catalyst at 80 C fo 40 min.

greater than 10%. Thus, sunflower oil apparently has a 1,3-random-2-random distribution of fatty acids.

The GRP-HPLC-FID triglyceride procedure proved useful for the qualitative and quantitative study of lipid interesterification reactions. Shown in Figure 2 is the compositional analysis of TGMS of the 1:1 blend (wt/wt) of soybean oil and fully hydro-palmitic acids among TGMS after interesterification. The results of quantitative analysis of these chromatograms is presented in Table 5, and indicate that potentially more oxidatively stable TGMS increased after interesterification; that is, TGMS which contain oleic compared to linoleic and linolenic acid and stearic compared to palmitic acids.

An example is the decrease in the LLLn-, LLL- and LLO-TGMS by 25%, 50% and 50%, respectively, as a result of interesterification. The OOO-TGMS increased five times. Mixed TGMS with stearic acid: SLS, SOS, LOS and LLS increased considerably. Thus,interesterification procedures are potentially useful for developing more stable shortenings, margarines and cooking oils (1,2).

GRP-HPLC-FID is a valid direct qualitative and quantitative method for TGMS in the study of vegetable oils and their reactions.

REFERENCES

1. Neff, W. E., Selke, E., Mounts, T. L., Rinsch, W., Frankel, E. N. and eitoun, M. A. M., Effect of Triacylglycerol Composition and Structures on Oxidative Stability of Oils from Selected Soybean Germplasm, J. Am. Oil Chem. In press.

2. Miyashita, K., Frankel, E. N., Neff, W. E. and Awl, R. A., Autoxidation of Polyunsaturated Triacylglycerols. III. Synthetic Triacylglycerols Containing Linoleate and Linolenate, Lipids, 25, 6314, 1990.

3. Frankel, E. N., Hydroperoxidation of Unsaturated Fatty Esters, in Oxygen Radicals, Biology and Medicine, Simic, M. G., Taylor, K. A. Ward, J. F. and von Sonntag, C., eds., Plenum Press, New York, 1988, p. 265.

4. Larsson, K., Physical Properties - Structural and Physical Characteristics, in The Lipid Handbook, Gunstone, F. D., Hardwood, J. L. and Padley, F. B., eds., Chapman and Hall, New York, 1986, p. 321.

5. Nurmela, K. V. V. and Satama, L. T., Quantitative Analysis of Triglycerides by High Performance Liquid Chromatography, using Non-Linear Gradient Elution and Flame Ionization Detection, J. Chromatogr., 435, 139, 1988.

6. Young, F. V. K., Poot, C., Biernoth, E., Krog, N., O'Neill, L. A. and Davidson, N. G. J., Processing of Fats and Oils, in The Lipid Handbook, Gunstone, F. D., Hardwood, J. L., Padley, F. B., eds., Chapman and Hall, New York, 1986, p. 208, 212.

7. Christie, W. W., Alternative or Complementary Methods for the Analysis of Molecular Species of Lipids, Reversed Phase Partition Chromatography, in Gas Chromatography and Lipids, The Oily Press, Glasglow, 1989, p. 242.

8. Phillips, F. C., Erdahl, W. L. and Schmit, J. A. and Privett, O. S., Quantitative Analysis of Triglyceride Species of Vegetable Oils by High Performance Liquid Chromatography via a Flame Ionization Detector, Lipids 19, 880, 1984.

9. Merritt, Jr., C., Vajdi, M., Kayser, S. G., Halliday, J. W. and Bazinet, M. L., Validation of Computational Methods for Triglyceride Composition of Fats and Oils by Liquid Chromatography and Mass Spectrometry, J. Am. Oil Chem. Soc., 59, 422, 1982.

10. Butterfield, R. O., Rohwedder, W. K., Bitner, E. D., Ernst, J. O., Wolf, D. J. and Dutton, H. J., Computers in the Lipid Research Laboratory, Prog. Lipid Res. 17, 93, 1978.

11. Vander Wal, R. J., Calculation of the Distribution of the Saturated and Unsaturated Acyl Groups in Fats, from Pancreatic Lipase Hydrolysis Data, J. Am. Oil Chem. Soc., 37, 18, 1960.

12. Stirton, A. J., Fat Splitting, Esterification and Interesteri- fication, Bailey's Industrial Oil and Fat Products, Swern, D., ed., Interscience Publishers, New York, 1964, p. 965.

13. El-Hamdy, A. H. and Perkins, E. G., High Performance Reversed Phase Chromatography of Natural Triglyceride Mixtures:Critical Pair Separation, J. Am. Oil Chem. Soc. 58, 867, 1981.

14. Steel, R. G. D. and Torrie, J. H., Principles and Procedures of Statistics, McGraw Hill, New York, 1960.

15. Blank, M. L. and Privett, O. S., Evaluation of Mathematical Distribution Methods for the Determination of Triglyceride Composition, Lipids 1, 27, 1966.

16. Fatemi, S. H. and Hammond, E. G., Glyceride Structure Variation in Soybean Varieties: 1. Stereospecific Analysis, Lipids 12, 1032, 1977.

17. DeLaRoche, I. A., Weber, E. J. and Alexander, D. E., Effects of Fatty Acid Concentration and Positional Specificity on Maize Triglyceride Structure, Lipids 6, 531, 1971.