Principal Component Analysis in Fast Atom Bombardment–Mass Spectrometry of Triacylglycerols in Edible Oils

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ABSTRACT: Positive-ion fast atom bombardment–mass spectrometry, with *m*-nitrobenzyl alcohol as matrix and a methanolic sodium iodide (NaI) solution, was used to characterize edible oils by their triacylglycerol composition. Cationized triacylglycerols gave characteristic spectra for olive (57 samples), sunflower (9 samples), soybean (9 samples), corn (12 samples), and peanut (10 samples) oils. Relative abundance of $[M + Na]^+$ ions showed relevant differences among the oils. The obtained data were analyzed with the aid of chemometrics by using principal component analysis (PCA) to evaluate the differentiation. Twelve variables were reduced to two principal components, accounting for 82% of the total variance. A two-dimensional plot of 97 PCA scores allowed the separation of the edible oils. The analysis is both fast and reproducible.

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KEY WORDS: Chemometrics, edible oils, FAB–MS, PCA, triacylglycerols.

The analysis of edible fats and oils by their triacylglycerol content has recently assumed a great importance as far as quality control and possible counterfeits of examined products. The principal methods of analysis are chromatographic, such as high-resolution gas chromatography or high-performance liquid chromatography (1–6). Mass spectrometry (MS) is considered an optional technique for these analyses; it is usually employed like a chromatographic detector, with the aim of obtaining more qualitative information (7,8). Different ionization techniques have been used to evaluate the molecular weight of triacylglycerols—field desorption (9), chemical ionization (10), californium-plasma desorption (11), electro spray (12), and fast atom bombardment (FAB) (13–15); among these, FAB–MS was investigated by Evans *et al.* (15).

In this paper, a statistical analysis was used to evaluate the possibility for differentiating the most common edible oils by their triacylglycerol FAB spectra. Data were analyzed with the aid of chemometrics by using principal component analysis (PCA) to evaluate the differentiation. PCA is usually used in chemometrics to reduce q variables that describe n samples, solving the eigenvalue–eigenvector equation of the correlation matrix (**R**) between the variables. The eigenvectors that explain the major part of the total variance are the principal components (16–21).

The aim of this work was to obtain fast and reproducible results. The characteristic features of the FAB–MS of the most common edible oils could allow the analyst to rapidly differentiate various oils and to verify the possible counterfeits that usually occur.

EXPERIMENTAL PROCEDURES

Sample preparation. Solvents and reagents were purchased from Aldrich Chemical Co. Ltd. (Steinheim, Germany). Samples of 57 commercial olive, 9 sunflower, 9 soybean, 12 corn, and 10 peanut oils were collected during 1993. Oil solutions were prepared by adding 3 mL chloroform to 100 mg of the sample. Methanolic sodium iodide solutions (30 mg/mL) were prepared daily.

FAB–MS. Analyses were carried out by laying 0.5 μL *m*-nitrobenzyl alcohol as matrix, 0.5 μL of the oil solution, and 1 μL of the NaI solution on a FAB target. Mass spectra were acquired with a Finnigan Mat 90 (Finnigan Mat, San Jose, CA) reverse-geometry mass spectrometer equipped with a Xenon FAB gun and a saddle field beam generator (Ion Tech, Teddington, United Kingdom). The spectra were obtained with Xe pressure (source) of 10^{-5} torr, 6 KeV energy, and ion current of 1 mA.

Chemometric analysis. Relative abundance of the ions was used directly to produce an autoscaled **Z** matrix for the calculation of the correlation matrix, **R**, which then was utilized as the starting matrix in PCA. Principal components (PCs) were determined by considering eigenvalues (λ_j) and associated eigenvectors ($\mathbf{v}_j$) calculated from the characteristic equation: $(\mathbf{R}-\lambda_j\mathbf{I})\mathbf{v}_j = 0$. To choose an appropriate number of PC ($p < q$) to represent q original variables, the criterion of explained variance was adopted. Correlations (s_{jk}) between the jth variables and the kth PC were calculated and represented vectorially. The $\mathbf{Y} = \mathbf{ZV}$ matrix ($n \times p$) of PC scores was estimated from the eigenvector matrix. A normal VARIMAX rotation of the eigenvectors matrix was also applied. Statistical analy-

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ses were carried out with a STATGRAPHICS 4.0 software package (STSC Inc., Rockville, MD) on an IBM clone computer.

RESULTS AND DISCUSSION

The observed ions in the high mass region of the spectrum (*m/z* 800–1000) were due to cationized species $[M + Na]^+$ that corresponded to the most important triacylglycerols present in the examined oils. The identification was done following the procedure described by Evans *et al.* (15): Linked scans (magnetic field/electric field constant) for the most important ions present in the spectra showed the daughters that corresponded to the $[M - RCOO]^+$ ions; the experiments were carried out without collisional activation, and, therefore, only the major daughters were observed and identified. Triacylglycerols present in small amounts could not be detected. The fatty acid composition of the triacylglycerols was simply deduced from the daughter ions, but it was not possible to obtain information about the acids' position—the identified triacylglycerols were considered the sum of all possible isomers.

Figure 1 shows a typical olive oil FAB spectrum. The following ions were identified: (*m/z* 879) $[POL + Na]^+$, (*m/z* 881) $[POO + Na]^+$, (*m/z* 883) $[POS + Na]^+$, (*m/z* 903) $[LLO + Na]^+$, (*m/z* 905) $[LOO + Na]^+$, (*m/z* 907) $[OOO + Na]^+$ and (*m/z* 909) $[SOO + Na]^+$ where PLL = palmitoyl–dilinoleoyl–glycerol; POL = palmitoyl–oleoyl–linoleoyl–glycerol; POO = palmitoyl–dioleoyl–glycerol; POS = palmitoyl–oleoyl–stearoyl–glycerol; LnLnLn = trilinolenonyl–glycerol; LnLnL = dilinolenonyl–glycerol; LnLL = linolenonyl–dilinoleoyl–glycerol; LLL = trilinoleoyl–glycerol; LLO = dilinoleoyl–oleoyl–glycerol; LOO = linoleoyl–dioleoyl–glycerol; OOO = trioleoyl–glycerol; SOO, stearoyl–dioleoyl–glycerol.

Figures 2–5 show the FAB spectra of the seed oils. Other ions identified were: (*m/z* 877) $[PLL + Na]^+$, (*m/z* 895) $[LnLnLn + Na]^+$, (*m/z* 897) $[LnLnL + Na]^+$, (*m/z* 899) $[LnLL + Na]^+$, and (*m/z* 901) $[LLL + Na]^+$.

The observed ions were considered of diagnostic value for the statistical analysis, for this kind of study it was necessary to include the abundance values of all ions corresponding to

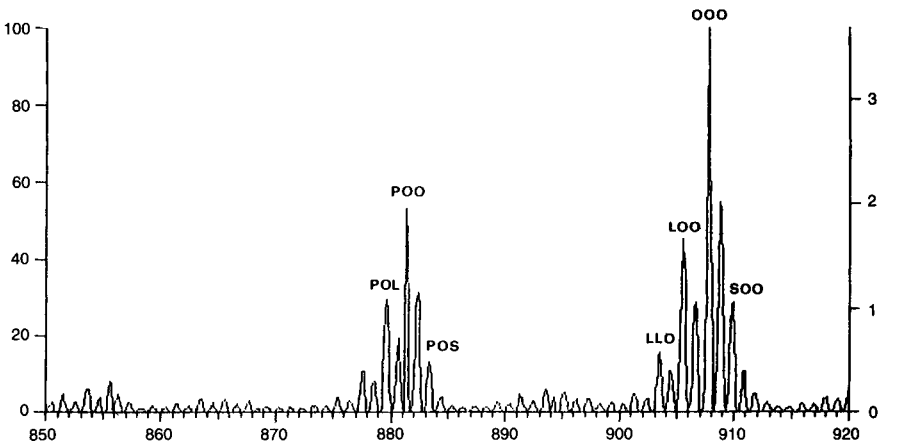

FIG. 1. Fast atom bombardment–mass spectrum of olive oil. POO, palmitoyl–dioleoyl–glycerol; POL, palmitoyl–oleoyl–linoleoyl–glycerol; POS, palmitoyl–oleoyl–stearoyl–glycerol; LLO, dilinoleoyl–oleoyl–glycerol; LOO, linoleoyl–dioleoyl–glycerol; OOO, trioleoyl–glycerol; SOO, stearoyl–dioleoyl–glycerol.

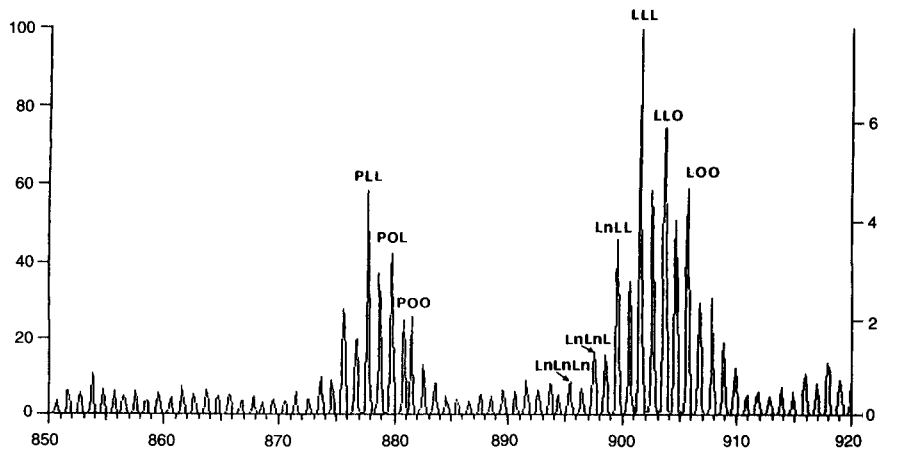

FIG. 2. Fast atom bombardment–mass spectrum of soybean oil. Abbreviations as in Figure 1; PLL, palmitoyl–dilinoleoyl–glycerol; LnLnLn, trilinolenonyl–glycerol; LnLnL, dilinolenonyl–linoleoyl–glycerol; LnLL, linolenoyl–dilinoleoyl–glycerol; LLL, trilinoleoyl–glycerol.

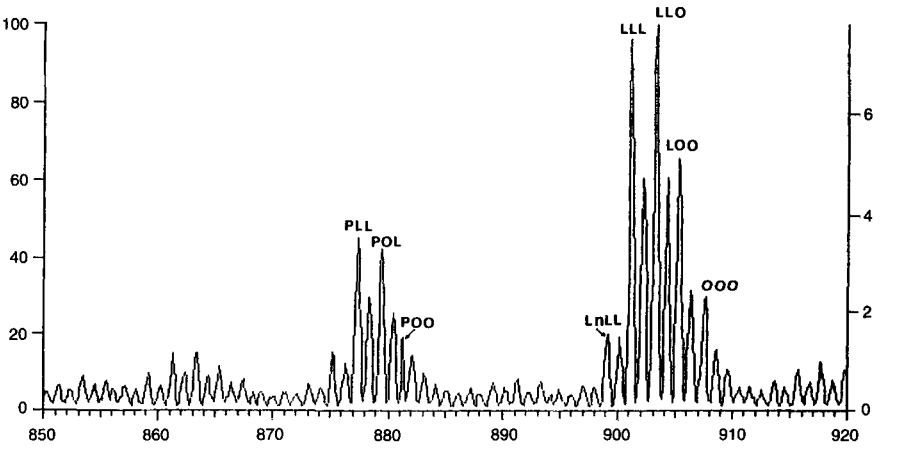

FIG. 3. Fast atom bombardment–mass spectrum of sunflower oil. Abbreviations as in Figures 1 and 2.

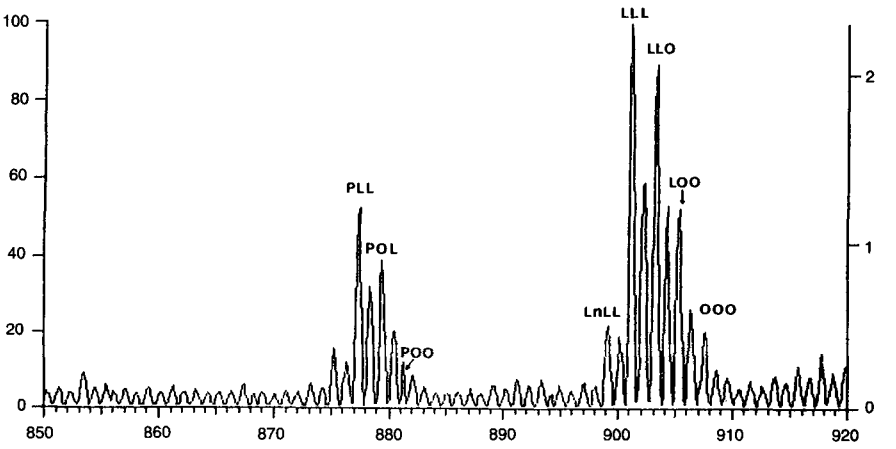

FIG. 4. Fast atom bombardment–mass spectrum of corn oil. Abbreviations as in Figures 1 and 2.

TABLE 1
Descriptive Statistics of the Relative Abundance of the Ions (a) Relative to the Triglycerides of Edible Oils[a]

	Olive (57 samples)			Soybean (9 samples)			Sunflower (9 samples)			Corn (12 samples)			Peanut (10 samples)		
m/z	Range of a	ā	|s|	Range of a	ā	|s|	Range of a	ā	|s|	Range of a	ā	|s|	Range of a	ā	|s|
877	9–36	19	6	38–88	62	14	26–70	43	16	36–78	60	12	27–50	39	6
879	22–69	42	13	16–80	48	17	18–86	45	25	36–86	57	17	57–85	71	9
881	31–84	60	10	8–47	25	10	13–64	29	21	12–61	29	14	39–73	57	10
883	10–28	18	4	7–18	10	4	5–32	14	10	4–20	11	6	13–33	26	6
895	4–19	8	3	6–15	10	3	4–16	8	4	5–15	8	3	10–20	15	3
897	3–11	6	2	7–27	16	6	5–11	8	2	5–13	9	3	8–18	11	3
899	2–11	5	2	19–54	42	11	14–30	20	5	5–29	21	6	6–20	12	5
901	4–20	9	3	90–100	99	3	69–97	82	9	44–100	87	16	16–46	29	10
903	12–40	24	8	72–100	85	10	100–100	100	—	76–100	95	8	46–90	64	14
905	37–80	56	13	51–77	61	8	45–84	65	11	51–100	68	13	83–100	95	6
907	100–100	100	—	21–46	32	8	21–67	37	16	21–97	35	21	64–100	88	14
909	22–38	30	4	11–25	16	4	7–21	12	5	6–25	14	6	28–40	33	4

[a]ā and |s|, mean and standard deviation (absolute value) of the relative abundance of the ions.

the different triacylglycerols in the data matrix **D**. Repeatability was estimated from ten olive oils that were tested daily for two weeks. Standard deviations on relative percentages of ions were: 1.60 (*m/z* 877), 3.43 (*m/z* 879), 3.03 (*m/z* 881), 2.32 (*m/z* 883), 1.72 (*m/z* 895), 1.37 (*m/z* 897), 0.89 (*m/z* 899), 0.55 (*m/z* 901), 2.26 (*m/z* 903), 2.66 (*m/z* 905), and 2.25 (*m/z* 909).

Results of descriptive statistics are reported in Table 1. The obtained data showed substantial differences among the oils. The most important ion in the olive oil spectra was always at

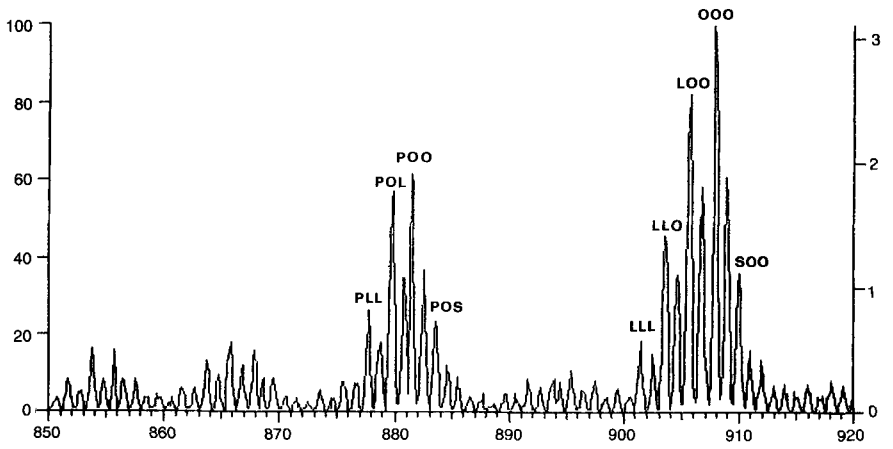

FIG. 5. Fast atom bombardment–mass spectrum of peanut oil. Abbreviations as in Figures 1 and 2.

TABLE 2
Correlation Matrix Between the Relative Abundance of the Ions Relative to the Triglycerides of Edible Oils[a]

	PLL	POL	POO	POS	LnLnLn	LnLnL	LnLL	LLL	LLO	LOO	OPOO	SOO
PLL	1.00	0.59	−0.49	−0.18	0.30	0.68	0.80	0.87	0.86	0.42	−0.79	−0.61
POL		1.00	0.23	0.45	0.60	0.41	0.18	0.22	0.40	0.73	−0.12	0.02
POO			1.00	0.73	0.25	−0.29	−0.64	−0.78	−0.69	0.08	0.82	0.78
POS				1.00	0.58	0.06	−0.38	−0.51	−0.35	0.31	0.57	0.70
LnLnLn					1.00	0.61	0.19	0.03	0.16	0.57	0.04	0.25
LnLnL						1.00	0.78	0.55	0.52	0.38	−0.48	−0.24
LnLL							1.00	0.87	0.74	0.20	−0.80	−0.67
LLL								1.00	0.93	0.19	−0.97	−0.86
LLO									1.00	0.44	−0.90	−0.77
LOO										1.00	−0.09	0.01
OOO											1.00	0.88
SOO												1.00

[a]PLL = palmitoyl–dilinoleoyl–glycerol; POL = palmitoyl–oleoyl–linoleoyl–glycerol; POO = palmitoyl–dioleoyl–glycerol; POS = palmitoyl–oleoyl–stearoyl–glycerol; LnLnLn = trilinolenonyl–glycerol; LnLnL = dilinolenonyl–linoleoyl–glycerol; LnLL = linolenonyl–dilinoleoyl–glycerol; LLL = trilinoleoyl–glycerol; LLO = dilinoleoyl–oleoyl–glycerol; LOO = linoleoyl–dioleoyl–glycerol; OOO = trioleoyl–glycerol; SOO, stearoyl–dioleoyl–glycerol.

m/z 907, and other characteristic peaks were at *m/z* 881 and 905.

The soybean oils showed the most important ions at *m/z* 901, 903, 877, and 905. In the sunflower and corn oils, however, a few differences were observed. The most important ions in these oils were at *m/z* 903, 901, and 905.

The peanut oils showed mass spectra with an ion relative abundance closer to the olive oils, with ions at *m/z* 905, 907, and 879 being the most important. From the data matrix **D**, an autoscaled **Z** matrix was obtained and used for the calculation of the correlation matrix, **R**, shown in Table 2. Some of the variables were mutually associated. The triacylglycerols were generally associated when two fatty acids were common. Thus, OOO is strongly correlated with SOO and POO; SOO with POO and POS; and LLL with PLL, LLO, and LnLL. The exception to this observed rule was LOO, which correlated with POL but not with OOO, POO, and SOO. In fact, OOO, POO, and SOO are typical olive oil triacylglycerols, whereas LOO is often the most important in seed oils.

The eigenvalues obtained from **R** were: 6.488, 3.334, 0.860, 0.434, 0.292, 0.191, 0.169, 0.082, 0.064, 0.038, 0.026, and 0.011. The first two eigenvectors explain about 81.9% of the total variance, and twelve variables can be reduced to 2 PC.

The values of s_{jk} are reported in Table 3. Five variables (PLL, LnLnL, LnLL, LLL, and LLO) are positively and three (POO, OOO, and SOO) are negatively correlated to PC1; Four variables are positively correlated to PC2 (POL, POS, LnLnLn, and LOO). An orthogonal rotation was tentatively applied to the s_{jk} matrix. The resulting rotated matrix shows factors that are nearly the same as those of the s_{jk} matrix; rotated factors are also reported in Table 3.

The differentiation between olive and seed oils is shown in Figure 6, which is a two-dimensional plot of 97 scores on the first two PCs. This figure shows that olive oils always have negative PC1 scores, although most of the seed oils have positive PC1 scores. In this exploratory plot, the olive oils are well separated from the seed oils; the peanut oils are close to the olive oils with no overlap and are clearly differentiated

TABLE 3
Matrix of the Correlations Between Variables and Principal Component (PC), and Orthogonally Rotated Factor Matrix[a]

	PC			Principal factor	
Variable	PC1	PC2	h^2	F1	F2
PLL	0.89	0.33	0.89	0.79	0.52
POL	0.27	0.83	0.76	0.08	0.87
POO	−0.79	0.46	0.83	−0.87	0.28
POS	−0.50	0.75	0.82	−0.66	0.62
LnLnLn	0.11	0.86	0.76	−0.09	0.86
LnLnL	0.63	0.51	0.65	0.50	0.63
LnLL	0.89	0.07	0.80	0.86	0.27
LLL	0.98	−0.07	0.97	0.98	0.15
LLO	0.94	0.12	0.89	0.89	0.33
LOO	0.28	0.75	0.63	0.10	0.79
OOO	−0.96	0.18	0.95	−0.97	−0.04
SOO	−0.84	0.38	0.86	−0.91	0.18

[a]h^2 is the communality. Abbreviations as in Table 2.

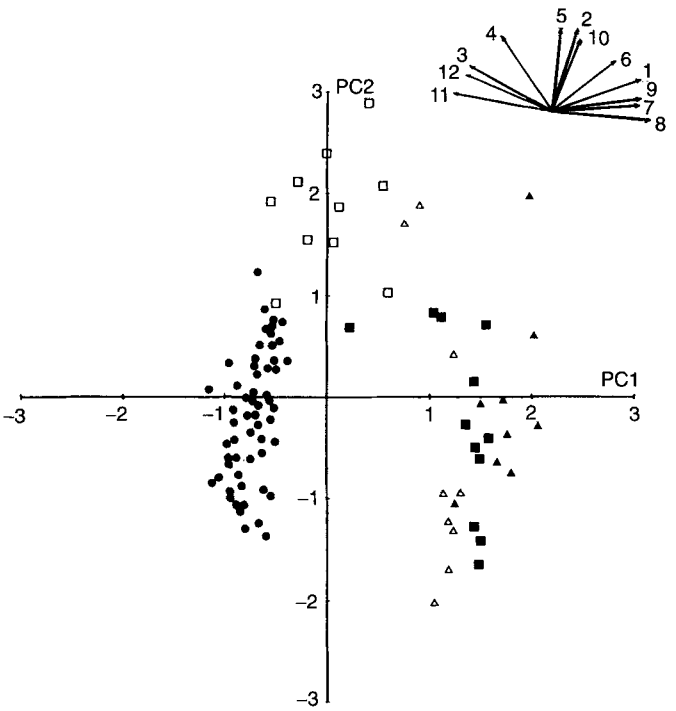

FIG. 6. Principal component (PC) scores of 97 samples of oils (●, olive, n = 57, ▲, soybean, n = 9; △, sunflower, n = 9; ■, corn, n = 12; □, peanut, n = 10). Association between PC and variable vector (1 = PLL, 2 = POL, 3 = POO, 4 = POS, 5 = LnLnLn, 6 = LnLnL, 7 = LnLL, 8 = LLL, 9 = LLO, 10 = LOO, 11 = OOO, 12 = SOO). Abbreviations as in Figures 1 and 2.

from the others. Corn, soybean, and sunflower oils give an appreciable separation, even if a few samples have intermediate characteristics.

The two-dimensional VARIMAX rotation plot doesn't provide an improvement in separation. The obtained results are extremely interesting because the different oils are well separated, and the analysis is fast (only a few minutes) as compared with other analytical methods.

These results allow the analyst to obtain a rapid differentiation of edible oils and provide an opportunity to discover those counterfeits that usually occur in olive oils.

REFERENCE

1. Peterson, B., O. Poldlaha and B.J. Toregard, *J. Am. Oil Chem. Soc. 58*:1005 (1981).
2. Motta, L., G. Carelli, P. Gamba, A. Gasparoli, C. Mariani, F. Camurati, O. Cozzoli and T. Zelinotti, *Riv. Ital. Sost. Grasse 63*:291 (1986).
3. Motta, L., G. Carelli, G. Lercker, C. Mariani, O. Cozzoli and T. Zelinotti, *Ibid. 64*:545 (1987).
4. Gilkinson, L.S., *Chromatographia 26*:181 (1988).
5. Marini, D., and F. Balestrieri, *Riv. Ital. Sost. Grasse 66*:11 (1989).
6. Cortesi, N., P. Rovellini and E. Fedeli, *Ibid. 67*:69 (1990).
7. Games, D.E., in *Applications of Mass Spectrometry in Food Science*, edited by J. Gilbert, Elsevier, Amsterdam, 1987, p. 206.
8. Sanders, R.A., and B.A. Charpentier, in *Proceedings of the 35th ASMS Conference on Mass Spectrometry and Allied Topics* (held May 24–29, 1987, in Denver, Colorado), American Society for Mass Spectrometry, Santa Fe, 1987, p. 1082.
9. Self, R., J. Eagles, F.A. Mallon and G.R. Fenwick, in *Mass Spectrometry of Large Molecules*, edited by S. Facchetti, Elsevier, Amsterdam, 1985, p. 221.
10. Currie, G.J., and H. Kallio, *Lipids 28*:217 (1993).
11. Pittenauer, E., G. Allmaier and E.R. Schmid, *Org. Mass Spectrom. 29*:108 (1994).
12. Sundin, P., C. Larsson, C. Wesen and G. Odham, *Biol. Mass Spectrom. 21*:633 (1992).
13. Keough, T., and R.A. Sanders, in *Proceedings of the 33rd ASMS Conference on Mass Spectrometry and Allied Topics* (held May 26–31, in San Diego, California), American Society for Mass Spectrometry, Santa Fe, 1985, p. 688.
14. Bartness, J.E., and L. Phillips, *Ibid.*, 1985, p. 963.
15. Evans, C., P. Traldi, M. Bambagiotti-Alberti, V. Giannellini, S.A. Coran and F.F. Vincieri, *Biol. Mass Spectrom. 20*:351 (1991).
16. Gnanadesikan, R., *Methods for Statistical Data Analysis of Multivariate Observations*, Wiley, New York, 1977.
17. Malinowsky, E.R., and D.G. Howery, *Factor Analysis in Chemistry*, Wiley, New York, 1980.
18. Muirhead, R.J., *Aspects of Multivariate Statistical Theory*, Wiley, New York, 1982.
19. Martens, M., and H. Russwurm, *Food Research and Data Analysis*, Applied Science, London, 1983.
20. Sharaf, M.A., D.L. Illman and B.R. Kovalsky, *Chemometrics*, Wiley, New York, 1986.
21. Kaufmann, P., in *Advances in Lipid Methodology–One*, The Oily Press, Ayr, 1992, p. 149.

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