Comparison Between Two Procedures for Stereospecific Analysis of Triacylglycerols from Vegetable Oils—I: Olive Oil

P. Damiani*, F. Santinelli, M.S. Simonetti, M. Castellini and M. Rosi
Istituto di Chimica Bromatologica, Facoltà di Farmacià, Università Studi, San Costanzo, 06100 Perugia, Italy

Two methods for stereospecific analysis of triacylglycerols are compared. Procedure A, based on stereospecific phosphorylation of *sn*-1,2-diacylglycerols to phosphatidic acids, and procedure B, based on separation of the diastereomeric 1,2(2,3)-diacylglycerol urethane derivatives by high-performance liquid chromatography on silica, were applied to olive oil triacyl-*sn*-glycerols. Statistical evaluation of the results showed good reproducibility, and Student's *t*-test indicates no statistical differences between the two considered procedures, although some small differences were observed and discussed. Fifteen samples of extra-virgin olive oil, produced in the same region (Umbria, Italy), were analyzed with the two considered procedures.

KEY WORDS: Diacylglycerols, olive oil, *sn*-1,2-diacylglycerol kinase, stereospecific analysis, triacylglycerols.

Stereospecific analysis of triacylglycerols has received much attention by many authors in the last few years. New procedures, essentially based on chemical steps, have been recently developed. The diacylglycerol enantiomers were separated by high-performance liquid chromatography (HPLC) either as diastereomers on a classical silica column (1,2) or as 3,5-dinitrophenylurethane derivatives on a chiral column (3,4). Some methods, involving chemical and enzymatic reactions, discussed in the 1960s, have been reconsidered with new enzymatic preparations. The enantiomeric *sn*-1,2(2,3)-diacylglycerols, isolated by preparative thin-layer chromatography (TLC), were transformed into phospholipid-like molecules, differentiated in turn by stereospecific action of phospholipase A or C (5,6). The same result was obtained by stereospecific phosphorylation of *sn*-1,2-diacylglycerols to *sn*-1,2-phosphatidic acids, an adenosine triphosphate (ATP)-dependent reaction catalyzed by *sn*-1,2-diacylglycerol kinase from *Escherichia coli* (7–10).

In this paper, two methods have been compared to determine reliability and repeatability. A sample of extra-virgin olive oil has been tested five times by each method. Then, fifteen samples of olive oil produced in Umbria (Italy) have been analyzed twice by the same two methods to determine fatty acid composition of *sn*-1, *sn*-2 and *sn*-3 positions.

The first method (procedure A) was based on partial deacylation of triacylglycerols with ethyl magnesium bromide, separation of *sn*-1,2(2,3)-diacylglycerols by TLC on boric acid-impregnated silica, stereospecific phosphorylation of *sn*-1,2-diacylglycerols to phosphatidic acids with a new *sn*-1,2-diacylglycerol kinase preparation from *E. coli*. The phosphatidic acids were then isolated by TLC on silica, and the fatty acid composition was determined by gas chromatography. The fatty acid composition of the *sn*-2 position was determined by partial hydrolysis of triacylglycerols to *sn*-2-monoacylglycerols *via* pancreatic lipase hydrolysis (11,12).

The second method (procedure B) was based on partial deacylation of triacylglycerols with ethyl magnesium bromide, derivatization of the total product with (S)-(+)-1-(1-naphthyl)ethyl isocyanate, purification of the products on octadecylsilyl solid-phase extraction columns and separation of the diastereomeric 1,2(2,3)-diacylglycerol urethane derivatives by HPLC on silica. The two fractions were then transesterified and analyzed by gas chromatography to determine the fatty acid composition (2).

Statistical evaluation of the results showed good reproducibility of both procedures, with coefficients of variation increasing by decreasing the percentage abundance of considered fatty acid. Student's *t*-test, (13) applied to the results obtained for the test sample, indicates that the two procedures give overlapping results with a satisfying confidence level for all the fatty acids considered.

MATERIALS AND METHODS

Extra-virgin olive oils were from the region of Umbria, Italy. All solvents and reagents were Analar or HPLC grades. Triacylglycerol samples (70 mg) were first purified on TLC plate (silica gel precoated plates, 20 × 20 cm, 250 μm; Sigma, St. Louis, MO) with hexane/diethyl ether/formic acid (80:20:2, vol/vol/vol) as developing solvent (14). The triacylglycerols (R_f = 0.64) were extracted from silica with diethyl ether (3 × 2 mL), and the organic extracts were pooled.

Triacylglycerols (10 mg) were dissolved in dry diethyl ether (2 mL), freshly prepared 0.5 M ethyl magnesium bromide in dry diethyl ether (0.5 mL) was added, and the mixture was shaken for 1 min before glacial acetic acid (50 μL) in hexane (5 mL) and water (2 mL) were added to stop the reaction (2,15). The organic layer was washed twice with water and dried over anhydrous sodium sulfate for 20 min. After evaporating the solvent in a stream of nitrogen at room temperature, the mixture of hydrolysis products was immediately used for subsequent steps.

*Stereospecific analysis of triacyl-*sn*-glycerols via sn-1,2-diacylglycerol kinase (procedure A).* This procedure has been described previously (10).

Synthesis of sn*-1,2-phosphatidic acids.* The hydrolysis mixture was applied to silica gel TLC plates impregnated with boric acid and developed with hexane/diethyl ether (1:1, vol/vol). The band containing the *sn*-1,2(2,3)-diacylglycerol fraction (R_f = 0.30) was scraped and extracted with diethyl ether (3 × 2 mL) (15). The extracts were pooled and concentrated with N_2 stream to ≅1 mL. Under continuous stirring, 0.1 mL of cardiolipin in methanol (5 mg/mL; Fluka, Buchs, Switzerland) was added to the solution, with subsequent ultrasonication for 1–2 min and solvent removal with N_2 stream. Twenty μL buffered solution of *sn*-1,2-diacylglycerol kinase (from *E. coli*, 1 mg/mL in 10 mM phosphate buffer pH 7.0, 20% glycerol, 2 mM β-mercaptoethanol, with defined specific activity; this specimen, 10.6 U/mg; Calbiochem Co., La Jolla, CA), 1 mL of buffer [605.7 mg tris(hydroxymethyl)aminomethane, 710.5 mg Na_2HPO_4, 584.4 mg NaCl, 203.3 mg $MgCl_2 \times 6H_2O$, 7.8 mg β-mercaptoethanol, 700 mg Triton X-100; pH adjusted to 6.6 with H_3PO_4; to 100 mL final volume with H_2O] and 100 μL aqueous solution of

*To whom correspondence should be addressed at Istituto di Chimica Bromatologica, Facoltà di Farmacia, Università Studi, San Costanzo, P.O. Box 346, 06100 Perugia, Italy.

Na_2ATP (8 mM; NBCO, Cleveland, OH) was added to the residue. After incubation at 40°C for 90 min under constant stirring, the reaction mixture was added of chloroform/methanol [1:1, vol/vol, made 1 ppm in butylated hydroxytoluene (BHT)] to stop the reaction and to extract the required products (2 × 1.5 mL). The combined extracts were concentrated to ≈1 mL, treated with anhydrous sodium sulfate and applied to silica TLC plate. The developing system was chloroform/methanol/ammonia 25% (65:25:5, vol/vol/vol). The band of the *sn*-1,2-phosphatidic acids, visualized with 2′,7′-dichlorofluorescein spray (0.1% in ethanol) (R_f range = 0.05–0.15), scraped, and the material obtained was introduced in a 15-mL test tube with a screw cap. The fatty acid constituents of the phosphatidic acids were transesterified for gas–liquid chromatography (GLC) analysis (see below).

Preparation of sn-*2-monoacylglycerols.* Triacylglycerols were subjected to hydrolysis with pancreatic lipase essentially as described previously (11,12). The *sn*-2-monoacylglycerols were extracted from scraped TLC bands and were methylated for gas-chromatographic analysis (see below).

Fatty acid analysis. The triacylglycerols, *sn*-1,2-phosphatidic acids and *sn*-2-monoacylglycerols were dissolved with 2 mL of pentane (made 1 ppm in BHT) and then 0.4 mL of 2 M KOH in anhydrous methanol was added. After 3 min, 3 mL water was added. The organic layer, separated by centrifugation, was dried over anhydrous sodium sulfate, then concentrated with an N_2 stream to ≈0.5 mL for GLC analysis. A Chrompack 9001 gas chromatograph (Chrompack International B.V., Middelburg, The Netherlands), equipped with a split/splitless injection system and a flame-ionization detector, was used for fatty acid analyses with a fused-silica column coated with Supelcowax 10™ (30 m × 0.25 mm i.d., d_f = 0.25 μm; Supelco, Bellefonte, PA). The temperature of the oven was maintained at 165°C for 3 min, then it was raised at 3°C/min to 240°C. Helium was the carrier gas. Quantitation was made by electronic integration by an EMI 80386 computer with MOSAIC integration software (Chrompack International B.V.).

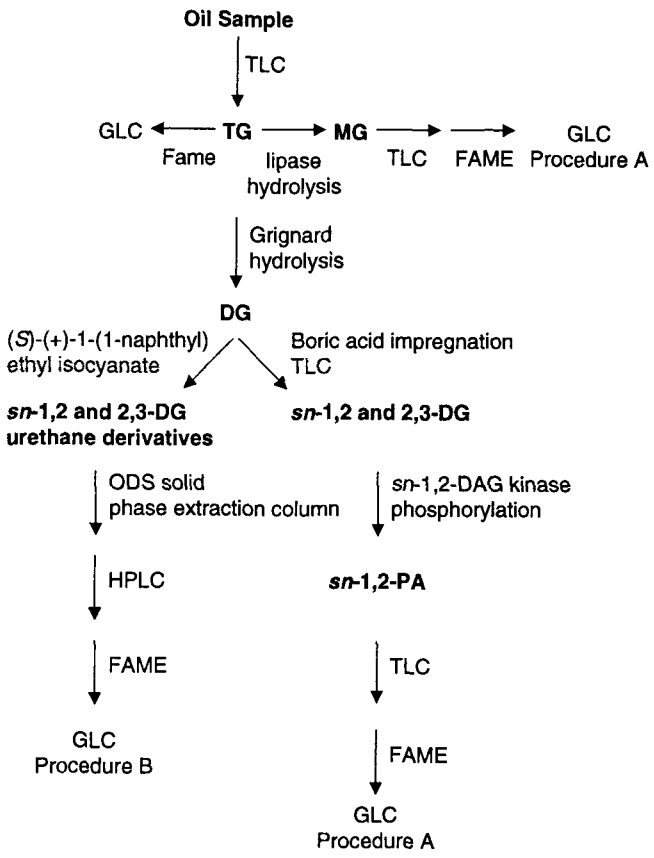
*Stereospecific analysis of triacyl-*sn*-glycerols via HPLC resolution of diastereomeric* sn*-1,2 and 2,3-diacylglycerols (procedure B) (2).* The mixture of hydrolysis products obtained by partial deacylation of triacylglycerols with a Grignard reagent were dissolved in dry toluene (1 mL), and (*S*)-(+)-1-(1-naphthyl)ethyl isocyanate (12.5 μL) and 4-pyrrolidinopyridine (4 mg) were added. The mixture was heated at 50°C overnight, and the solvent was removed in a stream of nitrogen the following day . Methanol/water (95:5, vol/vol; 6 mL) was added and warmed to dissolve the products. A Bond Elut™ ODS solid-phase extraction column (500 mg; Jones Chromatography, Hengoed, Wales) was solvated by passing 10 mL of this solvent through it. The reaction mixture was filtered through a small cotton-wool plug onto the column and washed through with a further 15 mL of solvent. The required products were then eluted with acetone (10 mL).

HPLC separation of the diastereomeric diacylglycerol derivatives was carried out with a Gilson Model 307 isocratic pump, a Gilson Model 115 UV-detector (Middleton, WI), equipped with a Rheodyne 7125 sample injection valve (Cotati, CA) and a Hewlett-Packard HP 3394 integrator (Palo Alto, CA). Two columns of silica gel (Hypersil 3 μm, 25 cm × 4.6 mm i.d.; HiChrom Ltd., Reading, United Kingdom) in series were utilized with 0.3% (vol/vol) *n*-propanol (containing 2% water) in hexane as mobile phase at a flow rate of 1 mL/min. The sample was injected in the minimum volume of hexane (5–10 μL). Detection was at 280 nm.

Fatty acid analysis. The methyl ester derivatives of the fatty acids from each fraction were prepared by sodium methoxide-catalyzed transesterification (2). The gas-chromatographic equipment and conditions were the same as indicated for procedure A.

RESULTS AND DISCUSSION

Stereospecific analysis of triacylglycerols could, in the near future, be a routine analytical test to characterize vegetable oils. Several procedures have been reported (2,3,5,7,10), but rarely have they been compared. In this work, two previously described procedures (2,10) were considered for statistical evaluation of the results obtained. An attempt to recognize sources of procedural errors also was made. As shown in Scheme 1, TLC purification and partial hydrolysis with ethyl magnesium bromide of triacylglycerols were common to both procedures to minimize potential differences occurring during these steps. As reported by other investigators (1–4,10,15), the Grignard approach to partial deacylated triacylglycerols is one of the most reliable methods to obtain representative diacylglycerols. The hydrolysis mixture was then separated into two fractions, used, respectively, for procedures A and B. Before gas-chromatographic analysis,



SCHEME 1

two different reaction types were used for fatty acid methylation. In fact, *sn*-1,2(2,3)-diacylglycerol urethane derivatives show lower reactivity toward transesterification of fatty acids to methyl esters with respect to other glyceridic molecule (2).

All steps of the procedures considered are reported in Scheme 1. First, the procedures were applied to an olive oil test sample. Fatty acid compositions of *sn*-1,2-phosphatidic acids (procedure A) and *sn*-1,2 and 2,3-diacylglycerol urethane derivatives (procedure B) are listed in Table 1. Each reported value is the average of five determinations. The *sn*-1,3-diacylglycerol derivatives were not collected. As indicated previously (2), they do not give reliable results for the composition of position *sn*-2 because of acyl migration occurring during the Grignard reaction. Both procedures are quite long because of the number of synthetic, enzymatic and chromatographic steps. The coefficients of variation obtained for the fatty acid composition of *sn*-1,2-phosphatidic acids are similar to those obtained for the *sn*-1,2 and 2,3-diacylglycerol derivatives with values increasing with a decrease in the percentage relative abundance of the considered fatty acid. In theory, the fatty acid composition of *sn*-1,2-phosphatidic acids and *sn*-1,2-diacylglycerol derivatives should be equal. Results are similar for palmitoleic and stearic acid. Oleic acid is slightly higher when it is determined with procedure B, and the difference is equally distributed between palmitic and linoleic acid (slightly lower) to give a total of 100%.

Coefficients of variation, ranging from 0.8 to 1.6 for oleic acid, mean that it is not rare to obtain percentage abundance differing of 1–2 percentage points when a sample is tested twice with the same procedure. It is difficult to demonstrate where the observed differences originate. Fatty acid compositions of triacylglycerols and *sn*-2-monoacylglycerols obtained *via* pancreatic lipase hydrolysis give highly reproducible results (10–12). The reproducibility of results relative to *sn*-2-monoacylglycerols thus obtained is based on the widely demonstrated 1,3-specificity of porcine pancreatic lipase (11,16). For these reasons we have not performed statistical treatment on *sn*-2-monoacylglycerol and triacylglycerol data.

Specificity or selectivity of the *sn*-1,2-diacylglycerol kinase enzyme for a particular diacylglycerol molecule has not been demonstrated (17), and, in applying the procedure to synthetic triacylglycerols, the results agreed with theoretical values (10). In procedure B, there are no potentially specific or selective steps. However, a complete resolution of the *sn*-1,2 and 2,3-diacylglycerol urethane derivatives by HPLC is necessary (2). This is easily monitored by the chromatographic trace.

The quantity of fatty acid methyl esters for the final gas-chromatographic analysis is low in both cases (2,10). The results obtained may be considered satisfying, but the fatty acid composition of positions *sn*-1, *sn*-2 and *sn*-3 in the triacylglycerol molecule is obtained by combining the composition of *sn*-1,2-diacylglycerol derivatives with those obtained for triacylglycerols and *sn*-2,3-diacylglycerol derivatives (procedure B), or by combining the composition of *sn*-1,2-phosphatidic acids with those obtained for the triacylglycerols and *sn*-2-monoacylglycerols (procedure A). Computation may amplify or minimize the observed differences, and the final result may be different sometimes (up to 3–4%).

Fatty acid compositions of *sn*-1,2-phosphatidic acids and *sn*-1,2-diacylglycerol urethane derivatives were compared by computing Student's *t*-test probabilities for the two sets of data derived from two different populations. For each fatty acid, two series of data were considered. The method used was that for two tails and heteroscedastic distributions. The values are shown in Table 2. For a confidence level of 95% and 8 degrees of freedom the maximum *t* value admitted is 2.306. All fatty acids give a result that is not significantly different when determined by procedures A and B. It is impossible to compare fatty acid composition of *sn*-2,3-diacylglycerol urethane derivatives obtained with procedure B with a similar result obtained *via* procedure A. On the other hand, the fatty acid composition of *sn*-2-monoacylglycerols, obtained *via* pancreatic lipase hydrolysis of triacylglycerols, is not comparable with the same result directly obtained *via* procedure B. The fatty acid composition of the *sn*-2 position from procedure B is obtained by subtracting those of *sn*-1 and *sn*-3 positions from the triacylglycerol fraction. In this case, the *sn*-2 position accumulates the maximum error by computation effects.

With procedure B, amplification of errors by computing effects could be minimized. The fatty acid composition of the *sn*-2 position directly obtained *via* pancreatic lipase hydrolysis can be combined with those obtained for triacylglycerols, and for *sn*-1,2 and 2,3-diacylglycerol urethane derivatives. In this case, the fatty acid composition of the *sn*-2 position is determined *via* pancreatic lipase hydrolysis. Fatty acid compositions of positions *sn*-1 and *sn*-3 are obtained by applying the following formulas:

$$sn\text{-}1 = (3 \times TG - 2 \times 2,3\text{-}DG + 2 \times 1,2\text{-}DG - MG)/2 \quad [1]$$

TABLE 1

Fatty Acid Compositions (mol% of the total) of *sn*-1,2-Phosphatidic Acids (1,2-PA) (procedure A) and *sn*-1,2(2,3)-Diacylglycerol Urethane Derivatives (1,2-DG and 2,3-DG) (procedure B) Obtained from the Olive Oil Test Sample

Fatty acid	1,2-PA			1,2-DG			2,3-DG		
16:0	8.6[a]	0.3[b]	2.7[c]	8.1[a]	0.4[b]	4.0[c]	10.6[a]	0.6[b]	5.1[c]
16:1[d]	0.6	0.3	41.8	0.5	0.1	24.9	0.5	0.1	18.8
18:0	1.2	0.1	8.2	1.2	0.1	9.1	2.1	0.2	10.4
18:1[e]	81.4	1.3	1.5	82.0	0.7	0.8	80.6	0.7	0.8
18:2	8.4	0.8	9.0	8.1	0.6	6.2	6.2	0.2	2.8

[a]Average of five determinations. [b]Standard deviation. [c]Coefficient of variation. [d]Sum of positional isomers n-7 and n-5. [e]Sum of positional isomers n-9 and n-7.

TABLE 2

Comparison of Fatty Acid Compositions (mol%[a]of the total) of *sn*-1,2-Phosphatidic Acids (1,2-PA) and *sn*-1,2-Diacylglycerol Urethane Derivatives (1,2-DG)

Fatty acid							*t*-Test Probability[b]	*t*-Test Value[c]
16:0	1,2-PA	8.69	8.67	8.47	8.17	8.85	0.05967	2.193
	1,2-DG	8.18	8.08	8.08	7.63	8.65		
16:1[d]	1,2-PA	0.71	0.71	0.10	0.71	0.60	0.88806	0.145
	1.2-DG	0.64	0.31	0.68	0.62	0.48		
18:0	1,2-PA	1.31	1.21	1.11	1.21	1.41	0.23456	1.286
	1,2-DG	1.06	1.18	1.05	1.34	1.15		
18:1[e]	1,2-PA	80.91	82.56	82.06	82.26	79.28	0.39895	0.891
	1,2-DG	82.76	81.72	81.53	81.28	82.85		
18:2	1,2-PA	8.28	8.57	7.56	7.86	9.76	0.57613	0.583
	1,2-DG	7.36	8.71	8.66	8.11	7.85		

[a]Indicated with two decimal points to minimize oversimplification introduced by round effect. [b]Probability for the two sets of data (obtained from two-tailed and heteroscedastic distributions) to be considered extracted from two different populations. [c]Inverse of the Student's *t*-distributions. [d]Sum of positional isomers n-7 and n-5. [e]Sum of positional isomers n-9 and n-7.

$$sn\text{-}3 = (3 \times TG - 2 \times 1,2\text{-}DG + 2 \times 2,3\text{-}DG - MG)/2 \quad [2]$$

where TG = triacylglycerols, DG = diacylglycerols and MG = monoacylglycerols.

A further result is obtained by combining the fatty acid compositions of triacylglycerols, *sn*-2-monoacylglycerols, again obtained *via* pancreatic lipase hydrolysis, and *sn*-1,2-diacylglycerol urethane derivatives; in this case, the compositions of *sn*-1, *sn*-2 and *sn*-3 positions are obtained as described for procedure A.

The fatty acid composition of *sn*-1, *sn*-2 and *sn*-3 positions obtained with procedures A and B are reported in Table 3. The same table also lists the results obtained by using the above-reported possible combinations (C and D).

Fifteen samples of olive oil were then analyzed with the two considered procedures. Table 4 shows the fatty acid composition of triacylglycerols, *sn*-2-monoacylglycerols, *sn*-1,2-phosphatidic acids and *sn*-1,2(2,3)-diacylglycerol urethane derivatives. For *sn*-1,2-phosphatidic acids and *sn*-1,2(2,3)-diacylglycerol urethane derivatives, each reported value is the average of two determinations. The obtained coefficients of variation were in agreement with those found for the test sample (Table 1). Fatty acid compositions of *sn*-1, *sn*-2 and *sn*-3 positions of the sample olive oils, obtained with procedures A and B, are reported in Tables 5 and 6.

Triacylglycerols from plant origin generally do not exhibit a markedly asymmetric distribution of fatty acids among the positions. Comparison between the results obtained for *sn*-1,2-phosphatidic acids and *sn*-1,2-diacylglycerol urethane derivatives of the sixteen analyzed olive oils confirms the tendencies (shown in Table 1) obtained for the test sample. Negative values were sometimes obtained for minor fatty acids.

TABLE 3

Fatty Acid Compositions (mol% of the total) of the Triacylglycerols (TG) from the Olive Oil Test Sample, the *sn*-2-Monoacylglycerols (MG) and *sn*-1, *sn*-2 and *sn*-3 Positions Obtained with Procedures A and B and Possible Combinations of Data

Fatty acid	TG	MG	*sn*-1 position				*sn*-2 position				*sn*-3 position			
			A[a]	B[b]	C[c]	D[d]	A[e]	B[f]	C[e]	D[e]	A[g]	B[h]	C[i]	D[j]
16:0	12.0	0.5	16.7	14.8	15.2	15.7	0.5	1.4	0.5	0.5	18.8	19.8	20.2	19.8
16:1[k]	0.7	0.5	0.7	1.1	0.9	0.6	0.5	0.1	0.5	0.5	0.9	0.9	0.7	1.0
18:0	2.0	0.0	2.4	1.8	2.1	2.3	0.0	0.6	0.0	0.0	3.6	3.6	3.9	3.7
18:1[l]	78.9	89.6	73.2	75.5	74.9	74.5	89.6	88.5	89.6	89.6	73.9	72.7	72.2	72.6
18:2	6.3	9.4	7.4	6.5	6.6	6.9	9.4	9.7	9.4	9.4	2.1	2.7	2.8	2.6

[a]2 × 1,2-PA − MG. [b]3 × TG − 2 × 2,3-DG. [c](3 × TG − 2 × 2,3-DG + 2 × 1,2-DG − MG)/2. [d]2 × 1,2-DG − MG. [e]MG. [f]3 × TG−(b + h). [g]3 × TG − 2 × 1,2-PA. [h]3 × TG − 2 × 1,2-DG. [i](3 × TG − 2 × 1,2-DG + 2 × 2,3-DG − MG)/2. [j]3 × TG − 2 × 1,2-DG. [k]Sum of positional isomers n-7 and n-5. [l]Sum of positional isomers n-9 and n-7.

TABLE 4

Olive Oil Samples: Fatty Acid Compositions (mol% of the total) of the Triacylglycerols (TG), the *sn*-2-Monoacylglycerols (MG), the *sn*-1,2-Phosphatidic Acids (1,2-PA) and the *sn*-1,2(2,3)-Diacylglycerol Urethane Derivatives (1,2-DG and 2,3-DG)

	Sample number	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
TG	16:0	12.0	10.4	11.1	10.7	12.5	12.4	11.6	11.8	11.8	12.4	12.3	10.9	12.4	11.3	11.4	12.4
	16:1[b]	0.7	0.7	0.5	0.5	0.9	0.8	0.6	0.6	0.8	0.7	0.8	0.6	0.5	0.6	0.8	0.6
	18:0	2.0	2.3	2.0	2.1	2.0	1.9	2.0	2.1	1.9	2.2	1.9	2.3	2.0	2.1	1.9	2.0
	18:1[c]	78.9	80.7	80.1	80.2	78.4	78.4	79.7	79.5	79.8	78.1	78.2	80.2	78.8	79.6	80.4	78.6
	18:2	6.3	5.8	6.3	6.5	6.2	6.5	6.1	6.0	5.7	6.6	6.8	6.0	6.3	6.4	5.4	6.4
MG	16:0	0.5	0.5	0.6	0.4	1.0	0.8	0.5	0.5	0.6	0.5	0.6	0.5	0.5	0.6	0.5	0.5
	16:1[b]	0.5	0.6	0.4	0.3	0.7	0.5	0.4	0.5	0.7	0.6	0.5	0.4	0.4	0.4	0.6	0.4
	18:0	0.0	0.1	0.1	0.0	0.0	0.2	0.3	0.1	0.6	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	18:1[c]	89.6	90.4	90.1	90.0	89.3	89.1	90.1	90.3	90.6	89.1	89.5	90.1	90.3	89.6	90.8	90.5
	18:2	9.4	8.4	8.8	9.3	9.0	9.4	8.7	8.6	7.5	9.7	9.3	8.9	8.7	9.3	8.0	8.5
1,2-PA	16:0	8.6	8.3	8.3	8.2	9.4	8.4	8.5	8.2	9.0	8.2	8.8	8.4	9.0	9.0	9.1	8.7
	16:1[b]	0.6	1.1	0.9	0.7	1.1	0.7	1.0	0.8	1.0	0.8	1.0	1.0	1.1	1.1	1.6	0.8
	18:0	1.2	1.5	1.4	1.4	1.4	1.2	1.6	1.3	1.3	1.6	1.3	1.7	1.7	1.6	1.7	1.4
	18:1[c]	81.4	80.0	81.1	81.3	79.5	80.7	81.9	82.6	80.3	82.4	78.7	81.9	80.5	80.7	79.9	82.3
	18;2	8.4	9.1	8.3	8.5	8.6	8.9	7.0	7.0	8.4	7.0	10.2	7.1	7.7	7.8	7.8	6.8
1,2-DG	16:0	8.1	7.7	7.2	7.3	8.7	7.9	7.8	8.3	7.9	8.4	8.3	7.4	9.4	8.4	8.5	8.6
	16:1[b]	0.6	0.6	0.4	0.7	1.1	0.9	1.0	1.0	1.1	0.6	1.0	0.5	0.7	0.5	0.4	1.1
	18:0	1.2	1.5	1.2	1.0	1.0	0.9	1.1	1.1	0.9	0.9	1.0	1.1	1.2	1.2	1.2	1.4
	18:1[c]	82.0	82.7	83.0	82.4	81.4	82.1	82.8	81.6	81.8	82.0	81.2	82.4	79.8	81.7	82.4	82.6
	18:2	8.1	7.5	8.2	8.6	7.9	8.2	7.4	8.0	8.3	8.1	8.5	8.7	9.0	8.2	7.5	6.3
2,3-DG	16:0	10.6	9.8	9.8	10.0	12.0	11.3	11.4	11.9	11.9	11.1	11.4	9.8	12.1	11.0	11.4	11.7
	16:1[b]	0.5	0.6	0.6	0.8	1.0	0.9	0.9	0.7	1.2	0.6	0.9	0.5	0.6	0.4	0.4	1.2
	18:0	2.1	2.2	2.0	1.9	1.8	1.9	1.8	2.0	1.7	1.9	1.8	1.9	1.8	2.1	2.1	2.3
	18:1[c]	80.6	81.7	81.7	81.2	78.6	79.4	79.2	79.6	79.4	78.9	79.3	81.5	78.9	80.5	80.8	79.9
	18:2	6.2	5.8	5.9	6.1	6.6	6.5	6.7	5.7	5.8	7.4	6.5	6.3	6.6	6.0	5.3	4.9

[a]Test sample (average of five determinations). [b]Sum of positional isomers n-7 and n-5. [c]Sum of positional isomers n-9 and n-7.

TABLE 5

Olive Oil Samples: Fatty Acid Positional Distribution (average of two determinations)

		Sample number	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
sn-1	16:0	A[b]	16.7	16.1	16.0	16.0	17.8	16.1	16.4	15.9	17.4	16.0	16.9	16.3	17.5	17.4	17.6	16.9
		B[c]	14.8	11.8	13.6	11.9	13.4	14.4	12.0	11.5	11.5	14.9	14.1	13.0	12.9	11.7	11.5	13.6
	16:1[d]	A	0.7	1.6	1.4	1.0	1.4	0.9	1.6	1.1	1.3	0.9	1.5	1.5	1.7	1.6	2.5	1.1
		B	1.1	1.0	0.3	0.0	0.7	0.6	0.0	0.4	0.1	0.9	0.6	0.8	0.4	1.0	1.7	−0.6
	18:0	A	2.4	2.9	2.6	2.7	2.8	2.2	2.9	2.5	2.0	3.0	2.5	3.2	3.3	3.0	3.3	2.7
		B	1.8	2.7	2.2	2.6	2.6	2.0	2.5	2.3	2.5	2.9	2.1	3.2	2.4	2.2	1.6	1.5
	18:1[e]	A	73.2	69.5	72.2	72.7	69.7	72.3	73.8	75.0	70.0	75.8	68.0	73.8	70.7	71.7	69.0	74.1
		B	75.5	78.8	77.0	78.3	78.0	76.5	80.7	79.1	80.5	76.4	75.8	77.6	78.7	77.8	79.6	76.1
	18:2	A	7.4	9.8	7.8	7.6	8.3	8.5	5.2	5.5	9.3	4.3	11.1	5.2	6.8	6.3	7.5	5.1
		B	6.5	5.8	7.0	7.2	5.3	6.5	4.9	6.6	5.5	4.9	7.4	5.3	5.6	7.2	5.6	9.4
sn-2	16:0	A[f]	0.5	0.5	0.6	0.4	1.0	0.8	0.5	0.5	0.6	0.5	0.6	0.5	0.5	0.6	0.5	0.5
		B[g]	1.4	3.5	0.8	2.6	3.9	1.3	3.5	5.1	4.4	2.0	2.4	1.8	5.8	5.0	5.5	3.6
	16:1[d]	A	0.5	0.6	0.4	0.3	0.7	0.5	0.4	0.5	0.7	0.6	0.5	0.4	0.4	0.4	0.6	0.4
		B	0.1	0.2	0.6	1.4	1.6	1.2	2.0	1.6	2.2	0.3	1.4	0.2	1.0	−0.1	−0.9	2.8
	18:0	A	0.0	0.1	0.1	0.0	0.0	0.2	0.3	0.1	0.6	0.1	0.1	0.1	0.1	0.1	0.1	0.1
		B	0.6	0.4	0.2	−0.5	−0.6	−0.2	−0.3	−0.1	−0.6	−1.2	−0.1	−1.1	−0.1	0.2	0.8	1.3
	18:1[e]	A	89.6	90.4	90.1	90.0	89.3	89.1	90.1	90.3	90.6	89.1	89.5	90.1	90.3	89.6	90.8	90.5
		B	88.5	86.6	88.9	86.5	84.8	87.7	84.9	84.1	83.0	87.5	86.5	87.2	80.8	85.7	85.1	89.1
	18:2	A	9.4	8.4	8.8	9.3	9.0	9.4	8.7	8.6	7.5	9.7	9.3	8.9	8.7	9.3	8.0	8.5
		B	9.7	9.3	9.5	10.0	10.4	10.0	9.8	9.4	11.0	11.4	9.7	12.0	12.4	9.2	9.5	3.2
sn-3	16:0	A[h]	18.8	14.7	16.6	15.6	18.6	20.2	17.8	19.0	17.3	20.7	19.3	15.9	19.1	15.8	16.2	19.6
		B[i]	19.8	16.0	18.7	17.5	20.1	21.3	19.2	18.8	19.5	20.3	20.3	17.9	18.3	17.1	17.3	19.9
	16:1[d]	A	0.9	−0.1	−0.3	0.2	0.6	1.0	−0.2	0.2	0.4	0.6	0.4	−0.1	−0.6	−0.2	−0.7	0.3
		B	0.9	0.9	0.6	0.1	0.5	0.6	−0.1	−0.1	0.2	0.9	0.4	0.8	0.2	0.9	1.6	−0.3
	18:0	A	3.6	4.0	3.4	3.7	3.3	3.3	2.9	3.8	3.2	3.6	3.2	3.7	2.6	3.3	2.4	3.3
		B	3.6	4.0	3.7	4.3	4.2	4.0	3.9	4.2	3.9	5.0	3.7	4.9	3.7	4.0	3.4	3.2
	18:1[e]	A	73.9	82.2	78.1	78.0	76.2	73.9	75.2	73.2	78.8	69.4	77.0	76.7	75.5	77.4	81.5	71.3
		B	72.7	76.8	74.4	75.8	72.4	71.1	73.4	75.2	75.9	70.3	72.2	75.8	76.9	75.3	76.6	70.7
	18:2	A	2.1	−0.8	2.3	2.6	1.3	1.6	4.4	3.9	0.3	5.7	0.1	3.9	3.4	3.6	0.6	5.5
		B	2.7	2.3	2.4	2.3	2.9	3.0	3.6	2.0	0.5	3.5	3.3	0.7	0.9	2.8	1.0	6.6

[a]Test sample (average of five determinations). [b]2 × 1,2-PA − MG. [c]3 × TG − 2 × 2,3-DG. [d]Sum of positional isomers n-7 and n-5. [e]Sum of positional isomers n-9 and n-7. [f]MG. [g]3 × TG − (c + i). [h]3 × TG − 2 × 1,2-PA. [i]3 × TG − 2 × 1,2-DG.

TABLE 6

Olive Oil Samples: Fatty Acid Positional Distribution[a] (average of two determinations)

		Sample number	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
sn-1	16:0	C[c]	15.7	14.8	13.8	14.1	16.3	14.9	15.0	16.1	15.2	16.3	15.9	14.3	18.3	16.1	16.5	16.7
		D[d]	15.2	13.3	13.7	13.0	14.9	14.7	13.5	13.8	13.3	15.6	15.0	13.6	15.6	13.9	14.0	15.2
	16:1[e]	C	0.6	0.6	0.5	1.1	1.5	1.3	1.5	1.4	1.5	0.6	1.5	0.6	1.0	0.5	0.2	1.8
		D	0.9	0.8	0.4	0.5	1.1	0.9	0.8	0.9	0.8	0.8	1.0	0.7	0.7	0.8	1.0	0.6
	18:0	C	2.3	2.9	2.2	2.1	1.9	1.6	1.9	2.1	1.3	1.6	2.0	2.0	2.2	2.3	2.2	2.7
		D	2.1	2.8	2.2	2.3	2.2	1.8	2.2	2.2	1.9	2.3	2.0	2.6	2.3	2.3	1.9	2.1
	18:1[f]	C	74.5	74.9	75.8	74.8	73.5	75.1	75.5	72.9	72.9	74.9	72.8	74.6	69.3	73.9	73.9	74.7
		D	74.9	76.9	76.4	76.5	75.8	75.8	78.1	76.0	76.7	75.6	74.3	76.1	74.0	75.8	76.8	75.4
	18:2	C	6.9	6.7	7.7	7.9	6.7	7.1	6.0	7.4	9.1	6.6	7.8	8.4	9.3	7.1	7.1	4.1
		D	6.6	6.2	7.3	7.6	6.0	6.8	5.4	7.0	7.3	5.7	7.6	6.9	7.4	7.2	6.3	6.7
sn-3	16:0	C[g]	19.8	16.6	18.9	17.7	20.5	21.3	19.3	18.6	18.7	20.3	20.2	18.0	17.3	16.9	16.1	18.9
		D[h]	20.2	17.5	18.9	18.6	21.5	21.6	20.7	21.0	21.4	21.0	21.2	18.5	21.0	19.3	19.8	21.4
	16:1[e]	C	1.0	0.8	0.7	0.1	0.3	0.3	−0.5	0.0	0.4	0.8	0.4	0.8	0.1	0.8	2.2	−0.4
		D	0.7	0.7	0.8	0.7	0.9	1.0	0.7	0.4	0.9	0.7	0.9	0.7	0.5	0.6	0.9	0.8
	18:0	C	3.7	4.1	3.7	4.3	4.1	3.9	3.6	4.1	3.9	5.0	3.6	4.8	3.3	4.1	3.2	2.9
		D	3.9	4.1	3.8	4.1	3.8	3.8	3.6	4.1	3.3	4.3	3.6	4.3	3.6	4.0	3.8	3.8
	18:1[f]	C	72.6	76.4	73.8	75.7	72.5	72.7	74.1	75.1	76.1	70.4	72.0	75.4	78.3	76.0	77.6	71.9
		D	72.2	74.9	73.8	74.1	70.1	70.4	70.9	72.1	72.1	69.6	70.7	74.4	72.2	73.3	73.7	70.0
	18:2	C	2.6	2.1	2.8	2.2	2.7	1.9	3.5	2.2	0.9	3.5	3.9	1.0	1.0	2.3	1.0	6.7
		D	2.8	2.8	2.8	2.6	3.5	3.3	4.1	2.4	2.3	4.3	3.5	2.2	2.7	2.7	1.8	3.9

[a]Position *sn*-2 already has been reported in Table 5. [b]Test sample (average of five determinations). [c](3 × TG − 2 × 2,3-G + 2 × 1,2-DG − MG)/2. [d]2 × 1,2-PA − MG. [e]Sum of positional isomers n-7 and n-5. [f]Sum of positional isomers n-9 and n-7. [g](3 × TG − 2 × 1,2-DG + 2 × 2,3-DG − MG)/2. [h]2 × 2,3-DG − MG.

REFERENCES

1. Laakso, P., and W.W. Christie, *Lipids 25*:349 (1990).
2. Christie, W.W., B. Nikolova-Damyanova, P. Laakso and B. Herslof, *J. Am. Oil Chem. Soc. 68*:695 (1991).
3. Itabashi, Y., A. Kuksis, L. Marai and T. Takagi, *J. Lipid Res. 31*:1711 (1990).
4. Takagi, T., and T. Suzuki, *J. Chrom. 519*:237 (1990).
5. Brockerhoff, H., *J. Lipid Res. 6*:10 (1965).
6. Myher, J.J., and A. Kuksis, *Can. J. Biochem. 57*:117 (1979).
7. Pieringer, R.A., and R.S. Kunnes, *J. Biol. Chem. 240*:2833 (1965).
8. Lands, W.E.M., R.A. Pieringer, P.M. Slakey and A. Zschocke, *Lipids 1*:443 (1966).
9. Bohnenberger, E., and H. Sandermann, *Biochim. Biophys. Acta 685*:44 (1982).
10. Damiani, P., M. Rosi, M. Castellini, F. Santinelli, L. Cossignani and M.S. Simonetti, *It. J. Food Sci. VI*:113 (1994).
11. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidmann and R.W. Riemenschneider, *J. Am. Oil Chem. Soc. 41*:693 (1964).
12. NGD, *Norme Italiane per il Controllo dei Grassi e Derivati*, edited by Stazione Sperimentale per le Industrie degli Oli e dei Grassi, Milano, 1989, Section C 46.
13. *Microsoft Excel User's Guide*, Book 2, edited by Microsoft Corporation, WA, 1992, p. 48.
14. Christie, W.W., M.L. Hunter and R.G. Vernon, *Biochem. J. 159*:571 (1976).
15. Christie, W.W., and J.H. Moore, *Biochim. Biophys. Acta 176*:445 (1969).
16. Sonnet, P.E., and J.A. Gazzillo, *J. Am. Oil Chem. Soc. 68*:11 (1991).
17. Walsh, J.P., L. Fahrner and R.M. Bell, *J. Biol. Chem. 265*:4374 (1990).

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