Technical

Chemometrics in Food Research: Relationships between Triglyceride Groups and Fatty Acid Composition in Olive Oil

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ABSTRACT

The values (mol %) of the triglyceride G_{NU} groups, each containing all the molecular species with the same carbon numer and unsaturation, are different for various oils; in this paper, their use for analytical purposes is therefore hypothesized. To this end, the values (mol %) of G_{NU} groups—with respective means, standard deviations and coefficients of variation—were first of all established using the 1,3-random-2-random distribution hypothesis on 50 olive oil samples; with these values, along with the relative data of the fatty acid composition, a computer study was carried out to obtain some regression models of use for estimating the G_{NU} values (mol %) of other olive oil samples. The evaluation of the regression models procedure (RMP) was carried out comparing the values obtained by such a method for 6 other commercial olive oils with those experimentally determined by Ag^+-TLC coupled with direct triglyceride GLC analysis. Moreover, these models were tested with the data of fatty acid composition of 4 different seed oils, providing often negative G_{NU} values, always different, however, from those of olive oil.

INTRODUCTION

Gas-liquid chromatography (GLC) has been frequently applied in the study of the composition of naturally occurring oils and fats; even if carried out with glass capillary columns, the gas-chromatographic analysis of pure triglycerides allows only the separation of the groups G_N, each containing all the triglyceride molecular species having the same carbon number but different unsaturations (1-28).

The triglyceride groups G_{NU}, each containing all the species with the same carbon number and unsaturation, can be separated by using a combination of Ag^+-TLC and GLC (13,16,18,29-31).

A further application of GLC for the analysis of triglycerides involves the separation of derivatives obtained by their oxidation and subsequent esterification; however, this procedure only gives the distribution of the individual saturated fatty acids in the glycerol moiety, while the unsaturated fatty acids are estimated together as azelaoglycerides (32-35). To obtain the distribution of both of the saturated and the unsaturated fatty acids a method has been devised which involves the separation of the triglyceride mixture into fractions differing in the total unsaturation (preparative Ag^+-chromatography), the subsequent oxidation of each fraction and, then, the GLC analysis of the azelaoglycerides contained in each fraction (31,36). However, none of these procedures is suitable for the resolution of all the glyceride molecular species and, least of all, for the separation of isomeric forms.

The values of all glyceride species can be calculated with the assumptions of Vander Wal (37) and Coleman (38), ("1,3-random-2-random distribution theory"), after the pancreatic lipase hydrolysis of the glycerides by the procedure described by Coleman (38) is carried out. (The cited theory is based on the equivalence of the fatty acid composition at the C_1 and C_3 positions, while some authors (39) demonstrated that this assumption is never completely true; consequently, the stereospecific analysis (39-41) should be employed for obtaining the real triglyceride composition; however, the less expensive and time-consuming procedure suggested by Vander Wal and Coleman is often preferred, especially when it provides results very similar to those of the stereospecific analysis (i.e., olive oil). A suitable combination of the data relative to these species provides the values of G_N and G_{NU} groups.

In the present study, we have perfected a series of multiple regression equations, using the values of the G_{NU} groups as described and the data of the fatty acid composition of 50 samples of olive oil from reliable sources. We have carried out this study for examining the possibility of determining the values of the triglyceride G_{NU} groups (and making use of these for some analytical purposes), using only fatty acid composition and the obtained equations. The validity of these relationships was evaluated by means of the multiple determination coefficient (R^2). These equations were then tested using the values of fatty acid composition of other samples of olive oil and some seed oils.

EXPERIMENTAL PROCEDURES

Samples and Their Characterization

This study was carried out on 50 samples of commercial olive oil from reliable sources. Each sample was at first examined by documented methods (42-45). The results obtained have allowed us to establish that all the samples were actually genuine olive oils. We also examined samples of soybean, sunflower, peanut and corn oils.

Triglyceride Fraction Separation

This was carried out for each sample according to methods described (46). The purity of the triglyceride fraction thus isolated was tested by thin layer chromatography (TLC); the Silica Gel G plate (20 × 20 cm layers 0.25-mm thick) used for this purpose was developed in benzene, ethyl ether, ethanol and glacial acid (50:40:2:0.2). For detection, the plate was sprayed with 2′,7′-dichlorofluorescein in ethanol (0.1% w/v).

Determination of Fatty Acid Composition (mol %) of the Triglyceride Fraction

The fatty acid composition of purified triglycerides was evaluated by GLC; for this purpose, the methyl esters were prepared as follows: an amount of 5-20 mg of triglycerides was refluxed with 10 mL of the mixture anhydrous CH_3OH/C_6H_6/conc. H_2SO_4 (75:25:1). After 30 min, 10-mL water was added, the methyl esters were extracted with ethyl ether (2-4 mL) and the obtained solutions directly analyzed. GLC was carried out with a Varian 3700 gas chromatograph, equipped with a flame ionization detector and a Varian CDS 111 C. The column (6 ft × 1/8 in.) packed with 20% LAC-886 on Chromosorb W 60/80, was operated isothermally at 180 C with a carrier gas flow (N_2) of 30 mL/min. Quantitative response factors were obtained from a mixture of known composition. The average fatty acid composition of triglycerides is reported in Table I.

Pancreatic Lipase Hydrolysis

This analytical procedure was carried out according to Coleman (38). The partial glycerides were separated on a thin layer (0.25 mm) of Silica Gel G, developed in petroleum ether (bp 30-50 C)/diethyl ether/formic acid 99% (70:30:1.5). The β-monoglyceride bands, detected with 2′-7′-dichlorofluorescein, were scraped off the glass TLC plate and refluxed as described above; the obtained methyl esters were also analyzed as indicated. The average fatty acid composition at the 2-position of triglycerides is reported in Table I. The fatty acid composition at the 1-3 positions was obtained using the following relationship:

$$\alpha\text{-}\alpha' \text{ (1-3)} = \frac{3T - \beta(2)}{2}$$

where α-α' (1-3) = mol % of each acid in the 1-3 positions, $\beta(2)$ = mol % of each acid in the 2-position, and T = mol % of each acid in the total triglycerides.

The triglyceride composition of each examined sample was obtained with the assumption of Vander Wal (37) and Coleman (38) and the fatty acid composition at the 2 and 1-3 positions.

G_N and G_{NU} Evaluation

The values (mol %) of each glyceride G_N group (G_{48}, G_{50}, G_{52} and G_{54}) of each olive oil sample were obtained by combining all the molecular species with the same carbon number without considering their unsaturation; the values (mol %) of each G_{NU} group ($G_{50.I}$, $G_{52.I}$, $G_{52.II}$, $G_{54.II}$, $G_{52.III}$, $G_{54.IV}$) were obtained by combining, for each sample, all the molecular species having both the same carbon number and the total unsaturation. In Tables II and III the means, SD and % CV for each group (G_N and G_{NU}, respectively) obtained from the 50 examined olive oil samples are reported; in the same Tables, the values (mol %) of G_N and G_{NU} groups of the 4 considered seed oils are also reported.

Statistical Analysis

This operation was carried out by means of a computer study using linear multiple stepwise regression models, performed with the BMDO2R library; for this purpose, the previously obtained values of the $G_{50.I}$, $G_{52.I}$, $G_{52.II}$, $G_{54.II}$, $G_{52.III}$, $G_{54.III}$, $G_{54.IV}$ (see preceding paragraph) were considered, along with the data of the relative fatty acid composition of total triglycerides of all the 50 olive oil samples. We thus obtained the regression models reported in Table IV.

TABLE I

Fatty Acid Composition[a] of the Triglyceride Fraction (mol %)

Samples	Palmitic acid		Palmitoleic acid		Stearic acid		Oleic acid		Linoleic acid		Linolenic acid		Arachidic acid		Eicosenoic acid		Behenic acid		Lignoceric acid	
	P_t	P_β	P'_t	P'_β	St_t	St_β	O_t	O_β	L_t	L_β	Ln_t	Ln_β	A_t	A_β	E_t	E_β	B_t	B_β	Li_t	Li_β
Olive oil (50 samples)																				
Mean	13.1	0.9	0.5	0.5	2.1	tr	79.3	91.5	4.7	7.1	0.2	tr	0.1	tr	tr	tr	–	–	–	–
SD	1.1	0.4	0.1	0.3	0.2	–	1.3	1.0	0.5	0.7	0.2	–	0.1	–	–	–	–	–	–	–
% CV	8.4	44.4[b]	20.0[b]	60.0[b]	9.5	–	1.6	1.1	10.6	9.9	100.0[b]	–	100.0[b]	–	–	–	–	–	–	–
Soybean oil	12.2	0.5	tr	tr	4.2	tr	23.6	24.5	54.3	69.8	5.6	5.1	–	–	–	–	–	–	–	–
Sunflower oil	6.7	0.4	0.1	tr	5.2	tr	27.1	25.1	60.1	74.3	0.2	tr	tr	tr	0.2	0.1	0.3	tr	–	–
Peanut oil	13.9	1.7	0.1	tr	4.6	0.4	48.2	52.6	25.2	45.2	–	–	1.0	tr	1.7	tr	3.6	tr	1.6	tr
Corn oil	11.8	0.8	0.1	0.2	3.0	0.2	25.9	25.4	57.6	72.7	1.4	0.8	0.1	tr	0.1	tr	–	–	–	–

[a]Symbol meaning:
P_t = total palmitic acid (mol %) in the triglyceride fraction.
P'_t = total palmitoleic acid (mol %) in the triglyceride fraction.
St_t = total stearic acid (mol %) in the triglyceride fraction.
O_t = total oleic acid (mol %) in the triglyceride fraction.
L_t = total linoleic acid (mol %) in the triglyceride fraction.
Ln_t = total linolenic acid (mol %) in the triglyceride fraction.
A_t = total arachidic acid (mol %) in the triglyceride fraction.
E_t = total eicosenoic acid (mol %) in the triglyceride fraction.
B_t = total behenic acid (mol %) in the triglyceride fraction.
Li_t = total lignoceric acid (mol %) in the triglyceride fraction.
P_β = palmitic acid (mol %) at the β-position of the triglyceride fraction.
P'_β = palmitoleic acid (mol %) at the β-position of the triglyceride fraction.
St_β = stearic acid (mol %) at the β-position of the triglyceride fraction.
O_β = oleic acid (mol %) in the β-position of the triglyceride fraction.
L_β = linoleic acid (mol %) in the β-position of the triglyceride fraction.
Ln_β = linolenic acid (mol %) in the β-position of the triglyceride fraction.
A_β = arachidic acid (mol %) at the β-position of the triglyceride fraction.
E_β = eicosenoic acid (mol %) at the β-position of the triglyceride fraction.
B_β = behenic acid (mol %) at the β-position of the triglyceride fraction.
Li_β = lignoceric acid (mol %) at the β-position of the triglyceride fraction.
[b]See text for explanation.

TABLE II[a]

Values (mol %) of the G_N Groups[b] of the Triglyceride Fraction

Sample	G_{48}	G_{50}	G_{52}	G_{54}
Olive oil (50 samples)				
Mean	tr	4.3	32.0	63.7
SD	–	0.7	1.9	2.6
% CV	–	16.3[c]	5.9	4.1
Soybean oil	tr	3.3	29.3	66.6
Sunflower oil	tr	1.0	18.0	80.2
Peanut oil	tr	4.4	28.1	48.1
Corn oil	tr	3.2	29.0	67.2

[a]In this table, the values of other G_N groups (G_{56}, G_{58}, etc.), although contained in the 4 seed oils considered, are not reported, since the olive oil (used in this work as test oil for statistical elaboration of its composition data) does not contain valuable molar percentages of groups different from those, reported in this table.

[b]Values obtained by combining in each group all the molecular glyceride species with the same carbon number without considering their unsaturation (derived from the Vander Wal-Coleman procedure application).

[c]See text for explanation.

Evaluation of the Regression Models Procedure (RMP)

The RMP was evaluated against a fully experimental procedure carried out on 6 commercial olive oil samples. For this purpose, the 6 samples were treated both according to the RMP described above and to direct GLC analysis (48) of the glyceride fractions (differing in the total unsaturation) previously obtained by preparative Ag^+-TLC (29) ($CHCl_3$ + C_2H_5OH [99:1] eluent) and separately quantitated by the chromotropic acid method (49). The results of this evaluation are reported in Table V. (The 4 considered seed oils were also treated according to the cited Ag^+-TLC/GLC procedure for obtaining experimental values of the glyceride groups, which proved quite similar to those obtained collecting the data of the glyceride molecular species determined by the Vander Wal-Coleman procedure and described in Tables II and III).

RESULTS AND DISCUSSION

The values (mol %) of the triglyceride groups G_N of olive oil are similar and comparable to the correlative values of

TABLE III[a]

Values (mol %) of Some of the Most Significant G_{NU} Groups[b] of the Triglyceride Fraction

Sample	$G_{50.I}$	$G_{52.I}$	$G_{52.II}$	$G_{54.II}$	$G_{52.III}$	$G_{54.III}$	$G_{54.IV}$
Olive oil (50 samples)							
Mean	3.6	1.2	26.3	4.3	4.2	49.8	8.6
SD	0.6	0.2	1.7	0.4	0.4	2.6	0.9
% CV	16.7[c]	16.7[c]	6.5	9.3	9.5	5.2	10.5
Soybean oil	0.8	0.6	3.6	1.1	10.1	4.8	13.4
Sunflower oil	0.3	0.4	2.6	1.6	6.9	7.4	19.7
Peanut oil	2.4	1.6	11.3	4.0	11.8	15.0	17.9
Corn oil	0.8	0.4	3.5	0.8	11.2	4.6	14.9

[a]In this table, the values of other G_{NU} groups, although contained in the 4 seed oils considered, are not reported, since the olive oil (used in this work as test oil for statistical elaboration of its composition data) does not contain valuable molar percentages of groups different from those reported in this table.

[b]Values obtained by combining in each groups all the molecular glyceride species having both the same carbon number and the total unsaturation (derived from the Vander Wal-Coleman procedure application).

[c]See text for explanation.

TABLE IV

Olive Oil: Regression Models for Some Groups vs Fatty Acid Composition of the Triglyceride Fraction[a]

1) $G_{52.I} = -1.189 + 0.086 \cdot P_t + 0.575 \cdot St_t$	$R^2 = 0.993$
(0.002)* (0.011)	SSE = 0.015
2) $G^b_{52.II} = -34.755 + 1.917 \cdot P_t + 0.467 \cdot O_t - 0.226 \cdot L_t$	$R^2 = 0.995$
(0.058) (0.050) (0.063)	SSE = 0.120
3) $G^b_{54.II} = -6.369 + 1.981 \cdot St_t + 0.083 \cdot O_t + 0.026 \cdot L_t$	$R^2 = 0.998$
(0.013) (0.002) (0.005)	SSE = 0.017
4) $G_{52.III} = 16.962 - 0.186 \cdot O_t + 0.430 \cdot L_t$	$R^2 = 0.834$
(0.020) (0.050)	SSE = 0.177
5) $G_{54.III} = -112.002 + 0.182 \cdot St_t + 2.017 \cdot O_t + 0.271 \cdot L_t$	$R^2 = 0.998$
(0.082) (0.014) (0.032)	SSE = 0.108
6) $G_{54.IV} = -18.885 + 0.232 \cdot O_t + 1.921 \cdot L_t$	$R^2 = 0.997$
(0.007) (0.016)	SSE = 0.059

[a]See Table I for explanation of some of the symbols used. R^2 = determination coefficient, SSE = standard error of estimation, and * = standard error of coefficient.

[b]Notice in particular that in the regression model of $G_{52.II}$ the sign in the O_t seems to contradict the theory, according to which it would carry a negative sign. In fact, the Bravais correlation coefficient between $G_{52.II}$ and O_t is -0.704. However, the sign reversal occurs because of the correlation between the other independent variables which defines a partial correlation coefficient between $G_{52.II}$ and O_t equal to 0.806. One can observe a sign which is contrary to expectations in the relationship between $G_{54.II}$ and O_t also (the Bravais correlation coefficient is negative (-0.114) but the partial correlation coefficient is positive (0.982). The correlation between the independent variables is not very high, however, and does not seem to cause any problems of multicollinearity (47).

TABLE V

Glyceride Composition of the 6 Commercial Olive Oils as Obtained Both with the RMP and the Experimental Procedure

Sample	Fatty acids (mol %)						Groups (mol %)											
							$G_{52.I}$		$G_{52.II}$		$G_{54.II}$		$G_{52.III}$		$G_{54.III}$		$G_{54.IV}$	
	C_{16}	C_{16}'	C_{18}	C_{18}'	C_{18}''	C_{18}'''	a	b	a	b	a	b	a	b	a	b	a	b
1	12.0	0.5	3.2	78.8	4.8	0.6	1.4	1.7	24.6	24.0	6.2	6.6	4.0	4.4	49.1	48.8	9.0	8.6
2	12.8	0.3	2.1	81.3	3.3	0.1	1.4	1.1	26.9	27.0	4.2	4.6	3.6	3.3	52.8	53.3	6.8	6.3
3	12.6	0.3	2.3	78.7	5.4	0.6	1.0	1.2	25.3	24.9	4.5	4.9	4.4	4.6	49.0	48.6	10.2	9.7
4	13.5	0.5	1.9	79.3	4.5	0.2	1.4	1.1	27.5	27.1	3.8	4.1	4.5	4.1	49.0	49.5	8.8	8.2
5	13.5	0.4	2.1	78.4	5.2	0.3	1.1	1.2	27.0	26.6	4.0	4.4	4.2	4.6	47.2	47.9	8.8	9.3
6	11.5	0.6	2.5	78.4	6.3	0.6	1.4	1.2	22.6	22.5	5.7	5.3	3.8	5.1	47.4	48.3	10.5	11.4
Mean	12.6	0.4	2.4	79.2	4.9	0.4	1.3	1.2	25.6	25.4	4.7	5.0	4.1	4.4	49.1	49.4	9.0	8.9
SD	0.8	0.1	0.5	1.1	1.0	0.2	0.2	0.2	1.9	1.9	1.0	0.9	0.3	0.6	2.0	2.0	1.3	1.7
% CV	6.3	25.0	20.8	1.4	20.4	50.0	15.4	16.7	7.4	7.5	21.3	18.0	7.3	13.6	4.1	4.0	14.4	19.1

[a]Values obtained by direct GLC analysis of the triglyceride fractions, differing in the total unsaturation, obtained from preparative Ag^+-TLC (see Evaluation of the Regression Models Procedure).
[b]Values obtained by RMP.

other considered seed oils (Table II). These values can be used to distinguish olive oil from only some of the cited pure seed oils (sunflower, peanut); it is therefore evident that they will not be significantly different from those of any olive oil/seed oil mixture. On the contrary, the values of the triglyceride groups G_{NU} of olive oil are generally distinctive and, since they are rather different from those of other seed oils (Table III), it is logically deductible that they should be different from those of olive oil/seed oil mixtures; this evidently proceeds from the different unsaturation of the fatty acids having the same carbon number. For an effective analytical utilization of G_{NU} groups, before all, it should be necessary to know the correct ranges of the values of all the G_{NU} groups of each type of oil, and this is only achievable by analyzing a large number of samples of each type.

In this paper, we have first considered 50 olive oil samples as described in Experimental Procedures, and as suggested by Vander Wal (37); the obtained data of glyceride types were then collected to give the values of G_N and G_{NU} groups, whose means, standard deviations and coefficients of variation (% CV) were also calculated. The % CV is an index of homogeneity for each type of the composition data of the 50 samples of olive oil (Tables I, II and III); the too-high values of % CV sometimes observed refer to the lowest composition data; therefore, these abnormal values have no significance as indexes of homogeneity.

The values of the G_{NU} groups were then utilized for the computer study, carried out to obtain the described regression models. These models, tested with experimental data of the fatty acid composition of 6 other samples of commerical olive oil, showed a considerable analytical significance; in Table V, the values of the G_{NU} groups obtained by RMP are compared with fully experimental data (Ag^+-TLC/GLC procedure) determined on the same 6 samples. Since the two series of data are very similar, this comparison proves the validity of the RMP as a rapid procedure for estimation of the triglyceride composition of olive oil.

However, the RMP must only be considered an initial approach relating to the application of statistical procedures to the estimation of the triglyceride composition of olive oil. In fact, since this work was carried out on 50 olive oil samples from a single zone, the regression models obtained are only effective for olive oils from the same zone; furthermore, we think these models can be perfected using a larger number of samples from the same source for statistical elaboration.

Therefore, we think that such a procedure might also be useful in typifying olive oils of well defined zones, if models formulated from analytical data of sure samples from various zones are available. In fact, depending on the composition, and thus on the type of cultivar and on pedoclimatic conditions, it is logical to hypothesize on a variability, although relative, of the data itself and therefore on the constants and coefficients of the regression equations mentioned above.

The RMP was also tested with experimental data of the fatty acid composition of the 4 considered seed oils: the regression equations proved useful in the analysis of olive oils, especially concerning the differentiation between olive and seed oils (see Table VI). In fact, from examining Table VI, we see that the values of the G_{NU} groups of each seed oil, calculated with the models relative to the olive oil and with the data of the fatty acid composition of these seed oils, are really quite different from those of olive oil and often also negative; furthermore, they are not comparable to the values of the same groups (reported in Table III) obtained from the data of the glyceride molecular species determined by the Vander Wal-Coleman procedure and as

TABLE VI

Values of Some G_{NU} Groups of the 4 Seed Oils Considered Obtained Using the Regression Models Relative to the Olive Oil Along with the Fatty Acid Composition Data of These Oils

Samples	Groups[a]					
	$G_{52.I}$	$G_{52.II}$	$G_{54.II}$	$G_{52.III}$	$G_{54.III}$	$G_{54.IV}$
Olive oil (average values, see Table III)	1.2	26.3	4.3	4.2	49.8	8.6
Soybean oil	2.3	-12.6	2.5	35.9	-48.9	90.0
Sunflower oil	2.4	-22.8	4.6	37.8	-40.1	102.9
Peanut oil	2.7	8.7	6.1	18.8	-7.1	40.7
Corn oil	1.6	-13.1	0.2	36.9	-43.6	97.8

[a]See text for explanation of the values reported in this table.

reported in Experimental Procedures. From Table VI it appears, moreover, that a few of these equations should even be able to detect any additions to olive oil, maybe even as low as 5%, of each of the seed oils examined; to verify this, further research will be carried out on suitable mixtures.

Similar models are also obtainable for other types of oil; they might be useful for some purposes, analogous to those described in this work for olive oil.

Finally, it is again proper to emphasize that the possibility of evaluation of triglyceride G_{NU} groups of a given oil (olive oil in this case) merely by the fatty acid composition data of total triglycerides and by means of suitable regression models saves considerable time and money, since some of the time-consuming analytical steps (enzymatic hydrolysis, extraction and various chromatographic processes) required in other methods are eliminated.

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