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Acylglycerol and Fatty Acid Components of Pulp, Seed, and Whole Olive Fruit Oils. Their Use to Characterize Fruit Variety by Chemometrics

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The contents of triacylglycerols and diacylglycerols in three kinds of olive fruit oils (pulp, seed, and whole fruit) were determined. The fatty acid composition and the quality ratios 1,2-diacylglycerols/1,3-diacylglycerols and 1,2-diacylglycerols/total diacylglycerols were also assessed. Seven major Italian olive varieties were considered. Results of univariate statistical analyses indicated that the above analytical parameters (glyceridic ratios excepted) were effective in discriminating between pulp and seed oils. The seed oil fraction did not determine any change in the glyceridic indices and the acylglycerol or fatty acid composition concerning the whole fruit oil (mixture of pulp and seed oil fractions), the weight (%) of seed (∼2%) being by far lower than the weight (%) of pulp (∼85%) (fruit weight basis). Based on the data of triacylglycerol or fatty acid composition, and using appropriate parametric or nonparametric multivariate statistics, the genetic origins (olive variety) of the three fruit oil kinds were characterized.

KEYWORDS: Olive pulp and seed oils; whole olive fruit oil; fatty acid and acylglycerol composition; glyceridic indices; chemometrics

INTRODUCTION

Virgin olive oil is a unique product because it is extracted by gentle physical procedures only, which results in a genuine fruit juice having excellent organoleptic and nutritional properties. Its richness in oleate makes it appropriate for direct human consumption, as well as for use in diets designed to reduce cardiovascular diseases (*1*, *2*).

Quality and typicality of virgin olive oil are primarily determined by genetic, climatic, and pedologic factors. Several typical virgin olive oils produced in the European Union's olive-growing countries have received a European protected origin denomination (POD) trademark or a European protected geographical indication (PGI) trademark; therefore, reliable multivariate statistical procedures should be studied to classify them in order to disclose commercial frauds. These typical oils are generally mixtures made up of a major oil variety and fixed proportions of some minor ones; thus, the studies to classify them are approached with the same procedures followed for monovarietal oils. Both saponifiable and unsaponifiable components can be selected for discrimination purposes (*3*−*6*).

In this study the triacylglycerol and fatty acid compositions, as well as the total triacylglycerol (TAG) and total diacylglycerol (DAG) contents, and the glyceridic indices (GI) of oils from pulp (mesocarp plus epicarp), seed (nonwoody part of the kernel), and whole olive fruit concerning seven different Italian olive cultivars were determined. The obtained analytical data were processed by parametric or nonparametric multivariate statistical methods in order to discriminate the olive variety. To our knowledge, such chemometric studies (excluding some approaches based on the fatty acid or triacylglycerol composition of the whole fruit oil) (*5*) have not been carried out so far.

MATERIALS AND METHODS

Chemicals. All reagents and solvents used in this work to extract the fruit oil kinds or to perform the analyses were mostly of chromatographic grade and were commercially available from either Carlo Erba (Milan, Italy), Fluka (Buchs, Switzerland), or Sigma-Aldrich Chemical (St. Louis, MO).

Olive Varieties Employed. Seven major Italian olive varieties (*Olea europaea* L.), including *leccino*, *dritta*, *caroleo*, *coratina*, *castiglionese*, *carboncella*, and *nebbio*, grown in the experimental olive grove of our institute (Istituto Sperimentale per l'Elaiotecnica, Pescara, Italy) were employed. These varieties give typical oils which have almost all received a European quality trademark and represent a large proportion of the bottled oil trade in Italy and in Europe (*7*). The selected trees (10 for each variety) from which the olive samples were collected by hand, had uniform characteristics, were 30 years old, and had been given drop irrigation and fertirrigation from the time of full bloom to fruit maturation (April to November).

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Sample Preparation of Fruit Oil Kinds. For each variety a 5 kg sample of olives, having normal ripeness extent and good sanitary state, was withdrawn at random. After manually removing stones from a 1-kg subsample of olives, seeds were obtained by carefully crushing the stones with a hammer. The pulp sample (~0.8 kg) was homogenized during 3 min by ultraturrax at medium speed. After adding some neutral alumina and anhydrous sodium sulfate to the homogenate to absorb the moisture, the sample was extracted in a Soxhlet apparatus with petroleum ether (40−70 °C) for 6 h (*8*). The seed sample (~0.2 kg) was ground separately in an ultraturrax for 5 min as described for olive pulp and extracted in a Soxhlet apparatus with petroleum ether (40−70 °C) for 6 h without drying (*8*). A 4-kg subsample of whole olive fruits was ground by a laboratory hammer crusher (Valpesana Srl, San Casciano Val di Pesa, Italy) and an aliquot (~0.8 kg) of the resulting paste was extracted using the Soxhlet method (*8*). The olive samples were stored frozen until extraction.

High-Resolution Gas Chromatographic (HRGC) Analysis of Fatty Acid Composition. Fatty acids were converted to fatty acid methyl esters (FAMEs) before analysis, and their relative composition was calculated as the % of the total fatty acids. A Mega Series model 5160 chromatograph (Carlo Erba, Milan, Italy), equipped with an on-column injection system and a flame-ionization detector (FID) was used. Separation was done on a fused-silica capillary column (25 m × 0.35 mm i.d., 0.25 μm film thickness, polyglycol type stationary phase, from Nordion Ltd., Helsinki, Finland), using hydrogen as the carrier gas (column pressure = 50 kPa). The oven temperature was programmed as follows: started at 120 °C, increased to 165 °C at a rate of 30 °C min^{-1}, held at this temperature for 25 min, increased to 200 °C at a rate of 5 °C min^{-1}, held at this temperature for 10 min, and increased to the end temperature of 220 °C at a rate of 5 °C min^{-1}. The injector and detector temperatures were 260 °C. The substance amount injected was 0.6 μL. No internal standard was used (*9*).

High-Performance Liquid Chromatographic (HPLC) Analysis of Triacylglycerol Composition. The content of individual triacylglycerols (expressed as % of the total triacylglycerols) was determined using a LDC 4 100 MS model system equipped with a Shodex RI Se-61 differential refractometer and a chromoject integrator (Thermo Separation Products, Schaumburg, IL). Separation was carried out by a reversed-phase column (4.5 × 250 mm, 5 μm) coated with supelcosil LC-18 (Supelco Inc., Bellefonte Park, PA). The mobile phase consisted of an acetone/acetonitrile (60:40, v/v) mixture, which was pumped at 1 mL min^{-1} at room temperature. Samples were prepared to a concentration of 50 mg oil per 1 mL of acetone, and sample solutions were filtered through 0.5-μm filters (Millipore SpA, Vimodrone, Milan, Italy). The substance amount injected was 10 μL. No internal standard was used (*9*).

Nuclear Magnetic Resonance (^{13}C NMR) Analysis of Total Triacylglycerols (TAG) and Total Diacylglycerols (DAG). The oil spectra were run in $CDCl_3$ (deuteriochloroform) (250 mg of oil/0.5 mL of $CDCl_3$) using a Bruker 300 spectrometer (Bruker Instruments, Karlsruhe, Germany) operated at 300 MHz. Free induction decays (FIDs) were acquired at 25 °C using a spectral width of 13000 Hz. The 131 K acquisition points were zerofilled to 256 K points. A 45° excitation pulse and a 20 s relaxation delay were employed to collect 256 scans. FIDs were processed before Fourier transformation by a Gaussian filter of 0.1 Hz Lorentzian narrowing and 0.15 Hz Gaussian broadening. Chemical shifts were relative to the signal of Me_4Si (tetramethylsilane) (*10*).

Statistics. A 7 × 3 factorial design (seven olive varieties × three fruit oil kinds) was used. The analytical data were processed by one-sided variance analysis (ANOVA). When a significant F value was found, means were separated using the Scheffé's post hoc pairwise test (*11*). Multivariate techniques (*12*, *13*), such as principal component analysis (PCA), hierarchical cluster analysis (HCA), soft independent modeling of class analogy (SIMCA), canonical discriminant analysis (CDA), and regularized discriminant analysis (RDA) were also used. The statistical method used depended on the characteristics of the data matrix, the chosen method being the one that gave both lower error rate and lower risk. Nonparametric multivariate methods (*12*), such as K nearest neighbor classification (KNN) and classification and regression tree (CART), were also used. To perform the multivariate analyses, four independent oil samples were analyzed for each olive variety and each fruit oil kind. The statistical software packages Statistica Release 6.0 (Statsoft Inc., Thulsa, OK) and Scan for Windows Release 1 (Minitab Inc., State College, PA) were used. A Pentium IV processor was used under Windows Xp Professional or Windows 98 2nd ed. operating system.

Table 1. Fatty Acid Composition (%) (as Determined by HRGC Method) of Oils from Pulp, Seed, and Whole Olive Fruit[a]

	type of oil		
type of fatty acid	pulp	seed	whole olive fruit
C16:0 (palmitic)	13.43 ± 1.21a	10.15 ± 1.02b	13.44 ± 1.15a
C16:1 (palmitoleic)	1.15 ± 0.07a	0.39 ± 0.04b	1.09 ± 0.08a
C17:0 (heptadecanoic)	0.11 ± 0.01a	0.10 ± 0.01a	0.12 ± 0.01a
C17:1 (heptadecenoic)	0.28 ± 0.02a	0.11 ± 0.01b	0.18 ± 0.02a
C18:0 (stearic)	2.45 ± 0.17a	2.87 ± 0.24b	2.56 ± 0.31a
C18:1 (oleic)	73.01 ± 7.55a	68.02 ± 6.37b	72.97 ± 6.89a
C18:2 (linoleic)	7.95 ± 0.83a	16.55 ± 1.84b	8.00 ± 0.63a
C20:0 (arachidonic)	0.47 ± 0.04a	0.51 ± 0.05a	0.45 ± 0.02a
C18:3 (linolenic)	0.66 ± 0.06a	0.42 ± 0.04b	0.58 ± 0.05a
C20:1 (eicosenoic)	0.28 ± 0.02a	0.59 ± 0.04b	0.39 ± 0.03a
C22:0 (behenic)	0.21 ± 0.02a	0.29 ± 0.02a	0.22 ± 0.01a
Σ SFA[b]	16.67 ± 1.55a	13.92 ± 1.25b	16.79 ± 1.30a
Σ MUFA[c]	74.72 ± 6.02a	69.11 ± 4.97b	74.63 ± 6.38a
Σ PUFA[d]	8.61 ± 8.92a	16.97 ± 1.63b	8.58 ± 0.76a

[a] Values (as % of total fatty acids) are means ± SD (n = 28) concerning oils from seven major Italian olive cultivars: *leccino, dritta, caroleo, coratina, castiglionese, carboncella,* and *nebbio.* For each cultivar and each fruit oil kind four independent samples were analyzed. Means within the same row with different superscripts are significantly different (Scheffé's test, $p \leq 0.05$). [b] SFA, saturated fatty acids. [c] MUFA, monounsaturated fatty acids. [d] PUFA, polyunsaturated fatty acids.

RESULTS AND DISCUSSION

The average analytical data and standard deviations for pulp, seed, and whole fruit oils, regardless of the olive variety, are presented in **Tables 1**, **2**, and **3**.

Fatty Acid Composition (%). Because the whole fruit oil is essentially made up of pulp oil, the features of these two oil kinds were very similar. It was observed that the three fruit oil kinds contained identical fatty acid species but with different concentrations (**Table 1**). Pulp and whole fruit oils were richer in individual monounsaturated fatty acids (eicosenoic acid excepted), as well as in total monounsaturated fatty acids (MUFA) essentially due to higher contents of oleic acid (the major fatty acid component found in the three fruit oil kinds). The two fruit oil kinds in question also were richer in total saturated fatty acids (SFA), essentially due to higher contents of palmitic acid (the major saturated fatty acid component found in the three fruit oil kinds), even though their content of stearic acid (another important saturated) was lower (**Table 1**).

Seed oil was richer in total polyunsaturated fatty acids (PUFA) (even though its content of linolenic acid was lower), because of higher contents of linoleic acid (the major fatty acid component of the PUFA fraction) (**Table 1**). These data were in general consistent with those produced by other authors, even though these used extraction solvents of another nature (*14*). Thus, from a nutritional viewpoint, seed oil fraction intake, unlike intake of the pulp or whole fruit oil fractions, results in a marked oxidation rate of the low-density lipoproteins (LDL), which contain thousands of fatty acid species coming essentially from the diet (*15*). Seed oil, however, according to some authors (*16*), in addition to being a minimal fraction, is only in part recovered with the current mechanical processing means (*9*), and studies are in progress to remove the kernel from the fruit

Table 2. Triacylglycerol Composition (%) (as Determined by HPLC Method) of Oils from Pulp, Seed, and Whole Olive Fruit[a]

type of triacylglycerol	type of oil		
	pulp	seed	whole olive fruit
trilinolein (LLL)	0.18 ± 0.02a	1.92 ± 0.16b	0.31 ± 0.02a
1,2-dilinoleoyl-3-oleoyl-glycerol (LLO)	1.90 ± 0.18a	7.85 ± 0.55b	1.79 ± 0.14a
1,2-dilinoleoyl-3-palmitoyl-glycerol (LLP)	1.42 ± 0.10a	2.26 ± 0.18b	1.45 ± 0.12a
1,3-dioleoyl-2-linoleoyl-glycerol (OLO)	13.92 ± 1.21a	20.27 ± 1.77b	14.32 ± 1.33a
1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol (POL)	6.83 ± 0.49a	7.93 ± 0.78b	7.31 ± 0.70a
triolein (OOO)	40.51 ± 4.17a	34.85 ± 3.22b	39.63 ± 3.43a
1,2-dioleoyl-3-palmitoyl-glycerol (OOP)	25.96 ± 1.61a	16.89 ± 1.54b	26.01 ± 2.51a
1,2-dipalmitoyl-3-oleoyl-glycerol (PPO)	4.51 ± 0.40a	2.57 ± 0.29b	4.23 ± 0.39a
1-stearoyl-2,3-dioleoyl-glycerol (SOO)	4.77 ± 0.43a	5.46 ± 0.41b	4.95 ± 0.52a

[a] Values (as % of total triacylglycerols) are means ± SD ($n = 28$) concerning oils from seven major Italian olive cultivars: *leccino, dritta, caroleo, coratina, castiglionese, carboncella,* and *nebbio.* For each cultivar and each fruit oil kind four independent samples were analyzed. Means within the same row with different superscripts are significantly different (Scheffé's test, $p \leq 0.05$).

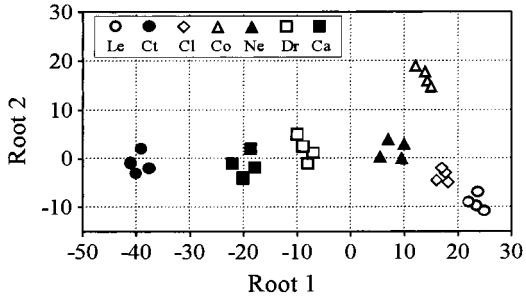

Figure 1. Classification of whole olive fruit oil samples from *leccino* (Le), *dritta* (Dr), *caroleo* (Cl), *coratina* (Co), *castiglionese* (Ct), *carboncella* (Ca), and *nebbio* (Ne) cultivars, based on their fatty acid composition, using canonical discriminant analysis (CDA) method.

prior to industrial extraction (*7*). Such an oil fraction could be recovered separately by laboratory procedures to classify unknown olive cultivars.

The olive variety (genetic store) factor affected the quantitative but not the qualitative fatty acid composition of each fruit oil kind (data not shown). In particular, the fruit oil kinds from the *caroleo* variety were richer in oleic acid and poorer in linoleic acid, whereas those from the *nebbio* and *leccino* varieties were poorer in palmitic acid.

The CDA classification method (*13*), based on the fatty acid composition data of the whole olive fruit oils, proved effective in discriminating between olive varieties (**Figure 1**). In fact, based on the Mahalanobis distance, all olive varieties were correctly classified by the two first roots (canonical functions). Along the first root were discriminated the *leccino*, *caroleo*, *coratina*, *nebbio*, and *dritta* varieties (positive half) as well as the *carboncella* variety (negative half), whereas along the second root was differentiated the *castiglionese* variety.

The RDA classification method (based on the fatty acid composition data of the whole fruit oils) also was effective in discriminating between olive varieties. This multivariate method seeks biased estimates of the covariance matrices in order to reduce their variance (*12*).

Triacylglycerol Composition (%). It was observed that pulp, seed, and whole fruit oils contained the same triacylglycerol species but with different concentrations, depending on the equivalent carbon number (ECN) of these components (**Table 2**). ECN of a triacylglycerol is defined as the number of carbon atoms present in its molecule minus two times the number of double bonds (*17*).

The three fruit oil kinds contained four major triacylglycerols, such as triolein (OOO), 1,2-dioleoyl-3-palmitoyl-glycerol (OOP), 1,3-dioleoyl-2-linoleoyl-glycerol (OLO), and 1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol (POL). In addition, they contained medium percentages of 1,2-dipalmitoyl-3-oleoyl-glycerol (PPO) and 1-stearoyl-2,3-dioleoyl-glycerol (SOO), and low percentages of 1,2-dilinoleoyl-3-palmitoyl-glycerol (LLP). 1,2-Dilinoleoyl-3-oleoyl-glycerol (LLO) was a major triacylglycerol for seed oil but a minor one for pulp and whole fruit oils. The OOO, OOP, and PPO triacylglycerol species (ECN = 48) were more abundant in the pulp and whole fruit oils, whereas the triacylglycerol species acylated with the linoleoyl chain (having a low ECN value), such as LLL (ECN = 42), LLO and LLP (ECN = 44), and OLO and POL (ECN = 46), were more abundant in seed oil. This also showed higher contents of the SSO species (ECN = 50). Such results were, in general, consistent with those obtained by other experimenters (*18*).

The differences in the relative triacylglycerol composition between pulp and seed oil can be explained by admitting that these substances are synthesized in the seed with the purpose of nourishing the embryo during the initial stages of germination, whereas the pulp triacylglycerols do not have any physiological purpose, so their fatty acid composition is more easily manipulated (*3*).

The olive variety factor affected the quantitative but not the qualitative triacylglycerol composition of each fruit oil kind. In particular, the fruit oil kinds from the *dritta* variety were richer in POL, whereas those from the *castiglionese* variety were richer in LLO and OLO, and those from the *caroleo* variety were richer in OOO but poorer in OLO.

It has been documented how the tactile-kinesthetic sensations from these fruit oil kinds are affected by the triacylglycerol composition factor, whereas their taste is affected by the fatty acid composition factor (markedly by the oleic acid/linoleic acid ratio) (*7*). Still, their saturated fatty acid components are by almost 100% distributed over the 1,3-positions of the glycerol backbone, whereas the unsaturated fatty acid components are more abundant in position 2 (*19*). Plant enzymes determine both the nature of fatty acid and the stereoselectivity in the acylation of glycerol (*10*).

On the basis of the triacylglycerol composition of the seed oils, three multivariate analysis methods, such as HCA (**Figure 2**), SIMCA, and KNN (**Figure 3**) were found to be effective in discriminating between olive varieties. HCA produces a hierarchy of partitions of objects such that any cluster of a partition is fully included in one of the clusters of the later partitions. Such partitions are best represented by a dendrogram (binary tree) (*13*). SIMCA is a biased version of the discriminant analysis technique and calculates a separate principal component for each class (*12*). KNN is a nonparametric method that searches for the K nearest neighbors of an object in the data

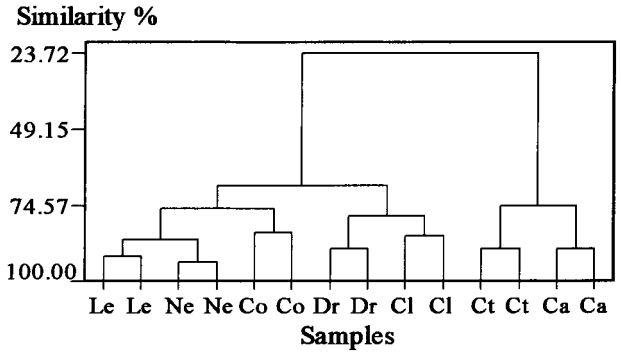

Figure 2. Dendrogram showing the clustering of olive seed oil samples from *leccino* (Le), *dritta* (Dr), *caroleo* (Cl), *coratina* (Co), *castiglionese* (Ct), *carboncella* (Ca), and *nebbio* (Ne) cultivars, based on their triacylglycerol composition.

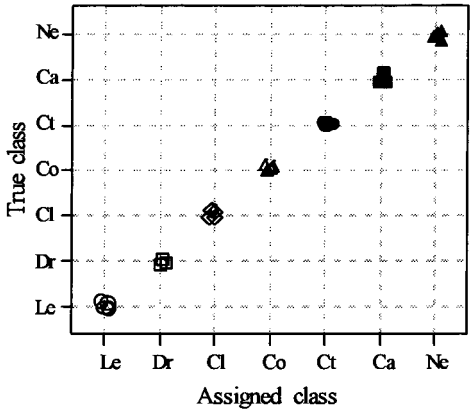

Figure 3. Classification of olive seed oil samples from *leccino* (Le), *dritta* (Dr), *caroleo* (Cl), *coratina* (Co), *castiglionese* (Ct), *carboncella* (Ca), and *nebbio* (Ne) cultivars, based on their triacylglycerol composition, using K nearest neighbor (KNN) classification method.

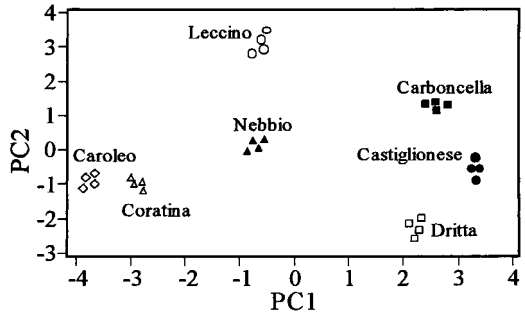

Figure 4. Score plot, by dimensions 1 and 2 from principal component analysis (PCA), of seed oil samples from seven olive cultivars, based on their triacylglycerol composition.

set, and estimates the class membership of this object from that of its neighbors (*12*).

Inspection of the plot in **Figure 4** revealed that the PCA technique, based on the triacylglycerol composition data of pulp oils, was another multivariate method capable of discriminating the olive variety. In fact, along the first dimension were discriminated the *dritta*, *castiglionese*, *carboncella*, and *nebbio* varieties (positive half), whereas along the second dimension were differentiated the *coratina* and *caroleo* varieties (negative half) as well as the *leccino* variety (positive half). Such a multivariate method, also referred as eigenanalysis method, is based on the maximum variance criterion, and calculates orthogonal linear combinations (principal component scores), whose coefficients are called loadings (*13*).

Table 3. Contents (%) of Total Triacylglycerols (TAG) and Total Diacylglycerols (DAG) (as Determined by ^{13}C NMR Method) and Values of the Glyceridic Ratios of Oils from Pulp, Seed, and Whole Olive Fruit[a]

	type of oil		
analytical parameter	pulp	seed	whole olive fruit
total TAG	93.63 ± 5.65a	96.88 ± 6.29b	93.56 ± 5.97a
1,2-DAG	1.51 ± 0.07a	0.93 ± 0.05b	1.76 ± 0.18b
1,3 -DAG	4.86 ± 0.39a	2.19 ± 0.14b	4.68 ± 0.27a
1,2-DAG/1,3-DAG ratio	0.31 ± 0.02a	0.42 ± 0.02a	0.38 ± 0.04a
1,2-DAG/total DAG ratio	0.24 ± 0.01a	0.30 ± 0.01a	0.27 ± 0.01a

[a] Values (as % of total glyceridic classes) are means ± SD ($n = 28$) concerning oils from seven major Italian olive cultivars: *leccino, dritta, caroleo, castiglionese, carboncella, coratina,* and *nebbio.* For each cultivar and each oil kind four independent samples were analyzed. Means within the same row with different superscripts are significantly different (Scheffé's test, $p \leq 0.05$).

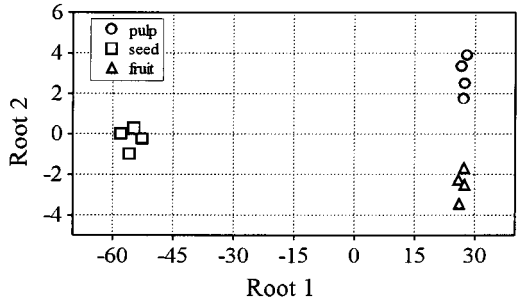

Figure 5. Classification of pulp, seed, and whole olive fruit oil samples from *leccino*, *dritta*, *caroleo*, *coratina*, *castiglionese*, *carboncella*, and *nebbio* cultivars, based on the triacylglycerol and diacylglycerol composition, using canonical discriminant analysis (CDA) method.

Total Triacylglycerols (TAG), Diacylglycerols (DAG), and Glyceridic Indices (GI). Examination of **Table 3** indicated that seed oil was significantly richer in TAG and poorer in DAG (1,2-DAG + 1,3-DAG). The glyceridic ratios (1,2-DAG/1,3-DAG and 1,2-DAG/DAG), which have been found to be qualitative indices, did not seem to discriminate the three fruit oil kinds ($p \leq 0.05$) (**Table 3**). The higher these indices, the better the quality (*20*). Each fruit oil kind had the glyceridic indices, total TAG, and total DAG affected by the olive variety factor. In particular, the fruit oil kinds from the *carboncella* and *castiglionese* varieties were poorer in total TAG and richer in total DAG, whereas those from the *caroleo* and *coratina* varieties were richer in total TAG and poorer in 1,3-DAG.

Inspection of the plot in **Figure 5** indicated how the CDA classification method (based on the data concerning TAG and DAG composition) was effective in discriminating between pulp, seed, and whole fruit oils. In fact, pulp and whole fruit oils were correctly classified along the first root (positive half), whereas seed oils were correctly classified along the second root.

ABBREVIATIONS USED

POD, protected origin denomination; PGI, protected geographical indication; TAG, triacylglycerols; DAG, diacylglycerols; GI, glyceridic indices; FAMEs, fatty acid methyl esters; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; ECN, equivalent carbon number; LDL, low-density lipoproteins; HRGC, high-resolution gas chromatography; HPLC, high-performance liquid chromatography; ^{13}C NMR, nuclear magnetic resonance; FID, flame ionization detector; FIDs, free induction decays; $CDCL_3$,

deuteriochloroform; PCA, principal component analysis; HCA, hierarchical cluster analysis; SIMCA, soft independent modeling class analogy; KNN, K nearest neighbor classification; CART, classification and regression tree; CDA, canonical discriminant analysis; RDA, regularized discriminant analysis; ANOVA, analysis of variance; SD, standard deviation.

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