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Diversity for olive oil composition in a collection of varieties from the region of Valencia (Spain)

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Abstract

Olive (*Olea europaea*) has a long history of cultivation in the Mediterranean region of Valencia (Spain) and many local varieties exist in the area. According to their economic importance, varieties are classified as National, Principal, Secondary, Local, Disseminate, and Minor. We have evaluated during four seasons the olive paste moisture content, fat content, and olive yield, and the olive oil acidity, peroxide index, K232 and K270 parameters, total phenolics, K225 parameter, and fatty acids content in 45 varieties from the Collection of Olive Varieties from the Region of Valencia. Considerable diversity existed among varieties for all traits studied, and the variety effect was much greater than the season effect. Wide ranges of variation have been found for most traits, with differences among varieties being of more than 10-fold for total phenolics. The coefficient of genetic variation and heritability values have been generally high, in particular for fatty acids content. A few varieties were found to present values outside the ranges established in the regulations for several olive oil composition traits, although in some cases, like a variety with above the limit content of oleic acid, they are of interest for breeding. Several correlations were found to be significant between K232 index and fatty acids profile, in particular with oleic acid. The values obtained for variety averages as well as the principal components analysis show that economically relevant varieties present a lower diversity for composition than varieties with low economic importance. In this respect, selection among traditional materials can be of interest to recover neglected varieties with specific composition profiles, as well as to identify sources of variation for breeding programmes. Discriminant analysis allowed a correct classification of 99.4% of samples, showing that composition profiles, in particular fatty acids content, is a powerful tool for chemometry and fingerprinting of olive oil. Overall, the results show that the wide diversity found in the collection studied, in particular in the less economically important varieties, is of interest for the selection and breeding of olive varieties with improved quality.

Keywords: chemometry, correlations, diversity, fatty acids, quality indexes, traditional varieties

1. Introduction

Olive (*Olea europaea* L.) is native from the Mediterranean region, where many wild populations exist (Breton et al., 2009). Olive was one of the first trees to be domesticated and there is evidence of its use and protocultivation in the Mediterranean area of Spain since early times around 7500-6500 years ago (Terral & Arnold-Simard, 1996). The natural and artificial selection, coupled with the effect of the other micro-evolutive forces, i.e., genetic drift, mutation, and genetic flow, together with the recombination resulting from sexual reproduction and subsequent clonal propagation of individuals with interesting characteristics, has resulted in the development of many traditional varieties (Bartolini, Prevost, Meseri, & Carignani, 1998). Some of these varieties spread to many olive cultivation areas, while others have only local importance, or have become neglected (Barranco & Rallo, 2000). Modern breeding resulting from directed crosses between traditional varieties has also allowed the development of new cultivars (Rallo, Barranco, de la Rosa, & León, 2008; León et al., 2011).

One of the areas will a longest history of olive tree cultivation is the region of Valencia, in the Mediterranean coast of Spain (Terral & Arnold-Simard, 1996; Barranco & Rallo, 2000). The total acreage devoted to olive oil production in 2010 in the region of Valencia was of 92.762 ha in regular plantations; also, an estimated 14250 scattered trees exist for olive oil production (MAAMA, 2011). As in other Mediterranean regions, olive oil is a fundamental piece of the local diet and cuisine of the region of Valencia. In this respect, olive oil is appreciated for its organoleptic characteristics, as well as for its high stability for cooking (Casal, Malheiro, Sendas, Oliveira, & Pereira, 2010; Inglese et al., 2011). In addition, demand is rising due to the ample and increasing evidence of the benefits for human health of olive oil consumption, which results from its high content in oleic acid and the presence of natural antioxidants such as phenolic compounds (Pérez-Jiménez, Ruano, Perez-Martinez, Lopez-Segura, & Lopez-Miranda, 2007).

Composition of olive oil is related to its physical, chemical, and organoleptic characteristics (Inglese et al., 2011). Some quality related parameters, like acidity, peroxide index, K232 and K270 parameters, total phenolics, and K225 parameter, together with the fatty acids profile are commonly studied and considered of importance for evaluating the quality of olive oil (Inglese et al., 2011). Several studies demonstrate that although olive oil presents a characteristic composition profile that allows distinguishing it from other plant oils (Jakab, Héberger, & Forgács, 2002; Brodnjak-

Vončina, Kodba, & Novič, 2005), a wide diversity can be found in composition among different varieties of olive oils (Barranco & Rallo, 2000; Vinha et al., 2005; Hannachi et al., 2008; Dıraman, 2010; Alba, Bisignano, Rotundo, Polignano, & Alba, 2012). Also, environment and season effects have been reported to affect olive oil composition (Cimato, 1990; Di Vaio, Nocerino, Paduano, & Sacchi, 2013).

At present, more than 70 olive accessions are conserved in the Collection of Olive Varieties from the region of Valencia. The varieties from this collection are classified in six categories according to their present importance: National, Principal, Secondary, Disseminate, Local, and Minor (Íñiguez, Paz, & Illa, 2001). National varieties are those with wide diffusion and found in regular plantations in all production areas of Spain; Principal varieties are those that are found in regular plantations and occupy an important acreage in the region Valencia and are dominant in at least one county; Secondary varieties are those that although can be found in regular plantations are not dominant in any county; Disseminate varieties are those that being spread in several counties they usually are not forming regular plantations; Local varieties are those that are found only in one or a few counties and usually are not forming regular plantations; Minor varieties are those that are poorly known and have a very marginal use with no regular plantations and that can only be found in scattered groups of trees or individual trees (Íñiguez, Paz, & Illa, 2001). Some of the varieties with lower economic importance characteristics could be recovered for commercial cultivation or for its use in breeding programmes (Aparicio & Luna, 2002; Caporale, Policastro, Carlucci, & Monteleone, 2006; Fabbri, Lambardi, & Ozden-Tokatli, 2009). Also, although most of the production of olive oil from the region of Valencia is in the form of blends, the marketing of monovarietal oils, in particular of local varieties, is increasing (Lerma-García et al., 2008).

Despite the interest of this collection, to our knowledge, no comprehensive study has been published reporting the characterization and evaluation of the composition of traditional varieties from the Spanish region of Valencia. The aim of the present study was to evaluate, using data from four seasons, parameters related to olive oil yield, quality indexes, and fatty acids composition in a collection of varieties from the region of Valencia. The results are of interest to describe the diversity existing in the collection, to assess the utility of composition data to discriminate among varieties, as well as to obtain information of relevance for breeding programmes.

2. Material and methods

2.1. Plant material

A total of 45 olive varieties from the Collection of Olive Varieties from the Region of Valencia (Spain) were used for the present study (Table 1). These varieties have been classified, depending on their economic importance into six established categories (Íñiguez, Paz, & Illa, 2001): National (8), Principal (5), Secondary (4), Disseminate (3), Local (14), and Minor (11). The varieties included in this collection have been characterized in detail and recognized as varieties that are distinct from others, uniform and stable (Íñiguez, Paz, & Illa, 2001). Therefore, potential synonymy or homonymy within the collection has been discarded. Comparison with varieties from other collections by means of internationally standardized descriptors and/or molecular markers may be useful to identify potential synonymies or homonymies with other minor olive varieties.

All the varieties were grown in the same plot in the Casa de Camp de Lliria-Casinos farm where the Collection of Olive Varieties from the Region of Valencia is maintained. Three trees, planted in 2000 in a block randomized design, were available for each variety.

2.2. Processing of samples

Olive fruits were harvested for four seasons (2005/06, 2006/07, 2008/09, and 2009/10). Harvesting was performed between the mid of November and end of December depending on the earliness of each variety. Fruits of the three trees of each variety were used to obtain an individual sample of around 5 kg for each season. This makes a total of four independent samples per variety. In order to make comparable the results of different season and varieties, fruits were selected for a uniform state of ripeness, which corresponded to the 3.5 ripening index according to Uceda & Frías (1975). The extraction process was performed using an Abencor MC2 (Ingenieria y Sistemas S.L., Sevilla, Spain) oil extraction system. After milling, the olive paste was fed into the thermomixer for 30 min and subsequently centrigufed at 3500 rpm for 1 min, after which the olive oil was recovered.

2.3. Traits measured

A total of 25 traits were measured, of which three corresponded to olive paste traits, six to olive oil quality indexes, 13 to individual fatty acids content, and three to fatty acids categories content.

For the olive paste we measured moisture content (%) by dessication at 105ºC for 24 h, fat content (%) by extraction with a Soxtec Avanti 2050 (Foss Tecator, Höganäs, Sweden) automatic extraction system, and olive oil yield (%). For the olive oil quality indexes, acidity (expressed as % of oleic acid), peroxide index (mEq $O_2 \cdot kg^{-1}$), and oil spectrophotometric indexes K232 and K270 were determined according to The Commission of the European Communities (1991, 2011); total phenolics were estimated according to the Folin-Ciocalteu spectrophotometric method (Singleton & Rossi, 1965) and expressed as $mg \cdot kg^{-1}$ of caffeic acid; and, bitterness spectrophotometric index K225 was measured according to Gutiérrez, Perdiguero, Gutiérrez, & Olías (1992).

For fatty acids determination, olive oil was subjected to transesterificantion with methanolic potassium hydroxide and n-heptane. The n-heptane extract was used to separate the fatty acid methyl esters using a Varian 3400 (Varian Associates, Walnut Creek, California, USA) gas chromatographer equipped with a Combi-Pal (CTC Analytics, Zwingen, Switzerland) autosampler and a flame ionization detector. The following fatty acids were determined: miristic acid (C14:0), palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0), palmitoleic acid (C16:1), margaroleic acid (C17:1), oleic acid (C18:1), gadoleic acid (C20:1), linoleic acid (C18:2), and linolenic acid (C18:3). Results for fatty acids content were expressed as percentage of the total fatty acid methyl esters present in the olive oil. From these determinations, the percentage of total saturated (SFA), monounsaturated (MUFA) and poliunsaturated (PUFA) fatty acids over the total fatty acids content were calculated.

2.4. Data analysis

Data were subjected to a two-way analysis of variance (ANOVA) to test the effects of the main factors season and variety. Normality of data and errors and homogeneity of variances were tested with the Kolmogorov-Smirnov and Bartlett tests, respectively (Little & Hills, 1978). Independence of errors was ensured by the random

distribution of the three plants from which the sample used were obtained. The total sum of squares was partitioned into the sums of squares for the season, variety, and residual effects and expressed in percentage over the total sum of squares. The coefficients of phenotypic variation (CV_P) and genotypic variation (CV_G) for each trait were estimated from the mean value and phenotypic variance (for CV_P) or genotypic variance (for CV_G) estimates obtained from the ANOVAs and expressed in percentage (Wricke & Weber, 1986). Broad-sense heritability (H^2) was estimated from phenotypic and genotypic variance estimates (Wricke & Weber, 1986).

For each trait, the mean and standard deviation were obtained for each category. Also, average standard errors for each mean were obtained from the ANOVA analyses (Little & Hills, 1978).

Phenotypic and environmental correlations between traits were calculated from correlations between variety means (phenotypic correlations) and between the residual effects of individual samples (environmental correlations), respectively (Jackson, 1994). The significance of phenotypic and environmental correlations was evaluated with the Bonferroni test at a significance level of P≤0.05. Bonferroni test was used in order to control the familywise error rate resulting from multiple pairwise (300 in our case) correlations (Hochberg, 1988).

Principal components analysis (PCA) was performed for standardized values of olive oil traits using pairwise Euclidean distances among variety means. Discriminant analysis was used to study the percentage of individual samples correctly classified according to the variety in the training population, which was composed by the whole set of data obtained. The forward stepwise procedure, with an F-to-enter and F-to-remove value of 4.0, was used for selecting the minimum subset of traits for obtaining the same number of correctly classified individual samples as in the model with all the traits (Tabachnik & Fidell, 2006). All statistical analyses were performed with Stagraphics Centurion XVI (StatPoint Technologies, Warrenton, VA, USA)."

3. Results

3.1. Analysis of variance and diversity parameters

The season and variety effects were significant (P≤0.05) for all traits, except in the case of the effect of season for acidity, C16:1, and C17:1 (Table 2). The variety

effect was the greatest contributor to the total variance for all traits, with the exception of moisture and acidity, in which the residual effect had the greatest contribution to the total sums of squares. Variety effects were much higher for the fatty acids content (always above 65%) than for the olive paste traits and olive oil quality indexes (always below 65%) (Table 2).

The average values for the moisture content of the collection was of 51.5%, while for the fat content it was of 22.5%, and for the oil yield of 17.6% (Table 2). The average acidity was of 0.40%, while for the peroxide index was of 5.51 mEq $O_2 \cdot kg^{-1}$. Regarding the K232 and K270 parameters, average values were of 1.62 and 0.12, respectively. Total phenolics content averaged 243 $mg \cdot kg^{-1}$ and the K225 parameter had a mean value of 0.18. The most important fatty acid was C18:1, with an average value of 70.94%, followed at a great distance by C16:0 (12.47%), C18:2 (11.10%), C18:0 (2.34%), and C16:1 (1.25%) (Table 2). The rest of fatty acids presented average content values below 1%, ranging from 0.01% (C14:0) to 0.75% (C18:3). Regarding the three categories of fatty acids, MUFA were predominant, with an average value of 72.69%, followed by SFA, with an average value of 15.47%, and finally by PUFA, with an average value of 11.84%.

The coefficient of phenotypic variation (CV_P) ranged between 9.62% for MUFA and 73.56% for the K225 parameter, while the coefficient of genotypic variation (CV_G) ranged between 6.94% for moisture content and 65.46% for C17:0 (Table 2). Compared to the other fatty acids, the CV_P and CV_G values of the predominant C18:1 fatty acid were low (10.31% and 10.14%, respectively). In general, for the fatty acids the CV_G values were closer to the CV_P values than for the rest of traits (Table 2). Broad-sense heritability (H^2) values ranged between 0.11 for acidity to 0.97 for C18:1, C18:2, MUFA, and PUFA. H^2 values for fatty acids content were generally higher than those of the olive paste and olive oil quality indexes (Table 2).

3.2. Variation among varieties

Important differences were observed among individual varieties for most of the traits. When considering the olive paste traits, we found that the moisture content ranged between 37.9% for Frantoio (Frn) and 62.0% for Matías (Mat) (Table 3). These two varieties also presented the highest and lowest values for fat content, with values of 30.8% for Frantoio (Frn) and 14.0% for Matías (Mat). These latter variety (Mat) also

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presented the lowest oil yield (8.3%), while the highest value was found in Villalonga (Vil; 26.0%) (Table 3). The commercially most important varieties had, in general, lower moisture values, and higher fat content and olive oil yield than the less important varieties (Table 3).

For the olive oil quality indexes considerable differences were also found among varieties for all traits. Acidity ranged between 0.17% for Aguilar (Agu) and 0.83% for Rogeta de Gorga (Rog) (Table 3). For the peroxide index value the range of variation was very high, from 1.5 for Dulce de Ayora (Dul) to 13.0 for Cuquellos (Cuq). K232 and K270 parameters presented a lower variability, with ranges going from 1.17 for Dulce de Ayora (Dul) to 2.21 for Rogeta de Gorga (Gor) for K232, and from 0.08 for Borriolenca (Bor) to 0.21 for Sollana (Sol) for K270. The total phenolics content and K225 parameter were very variable; the total phenolics content ranged from 55 mg·kg^{-1} for Carrasqueta de Ayora (CaA) to 646 mg·kg^{-1} for Sollana (Sol), and the K225 parameter ranged from 0.05 for Borriolenca (Bor) to 0.52 for Sollana (Sol) (Table 3). For the acidity, eight out of 13 varieties (62%) from the National and Principal categories and 11 out of 25% (44%) of the Local and Marginal categories had values below the general mean. For the rest of traits no appreciable differences were found depending on the type of variety.

Values for the minor saturated C14:0, C17:0, C18:0, C20:0, C22:0, and C24:0 fatty acids were low in all the varieties when compared with the major saturated fatty acid C16:0. In general, a wide range of variation among varieties was found for the minor fatty acids. For the C16:0 saturated fatty acid its content ranged between 7.37% for Lloma (Llo) to 20.38% for Datilera de Caudiel (Dat) (Table 4).

Regarding monounsaturated fatty acids, the C18:1 fatty acid was, by far, the most abundant in all varieties, and ranged from 56.8% in Rogeta de Gorga (Gor) to 84.2% in Valentins (Val) (Table 4). Three varieties, namely Aguilar (Agu), Lloma (Llo), and Carrasqueña de la Cañada (CaC) presented values of C18:1 above 80%; also, three varieties presented C18:1 values below 60%: Rogeta de Gorga (Gor), Blanqueta Gorda (BlG), and Datilera de Caudiel (Dat). For the minor monosaturated fatty acids C16:1, C17:1 fatty acids there was a wide range of variation, while for C20:1, it was relatively small (Table 4).

The major poliunsaturated fatty acid was C18:2 and was very variable, with values ranging from 3.32% in Aguilar (Agu) to 22.85% in Gileta (Gil) (Table 4). The

other poliunsaturated fatty acid measured (C18:3) presented much lower values than C18:2 in all varieties, and the variation presented was much lower (Table 3).

When considering the three categories of fatty acids (SFA, MUFA, and PUFA), the SFA content ranged from 10.51% in Lloma (Llo) to 23.74% in Datilera de Caudiel (Dat), the MUFA from 58.79% in Rogeta de Gorga (Rog) to 85.56% in Valentins, and the PUFA from 3.92% in Aguilar (Agu) to 23.74% in Gileta (Gil) (Table 4). The varieties with highest and lowest values for these categories of fatty acids were coincident with the varieties having the highest and lowest values for the major individual acids from each of these categories.

3.3. Correlations among traits

A total of 30 phenotypic correlations were significant according to the Bonferroni test at a significance level of P≤0.05 (Table 5). For 23 out of 30 of these phenotypic correlations, significant environmental correlations were also detected, in all cases having the same sign than the phenotypic correlations. The three olive paste traits studied were interrelated, with negative values for the phenotypic and environmental correlations between moisture on one hand and fat content and oil yield in the other, and positive values for the correlations between fat content and oil yield (Table 5). Regarding olive oil quality traits, K270 presented positive significant values for the phenotypic and environmental correlation with K232 and K225, and also a significant positive phenotypic correlation with total phenolics. The latter also presented significant positive phenotypic and environmental correlations with K225 (Table 5).

Regarding correlations involving individual fatty acids, C16:0 presented a significant positive phenotypic correlation with C16:1, and a negative phenotypic and environmental correlation with C18:1 and C20:1 (Table 5). The C17:0 and C17:1 fatty acids presented positive values for the phenotypic and environmental correlations. Significant positive values for the phenotypic and environmental correlations were found for C18:0 and C20:0, C20:0 and C22:0, and C22:0 and C24:0. Also, negative significant phenotypic and environmental correlation values were detected between C18:1 and C18:2. For fatty acid categories, we found that MUFA presented significant negative phenotypic and environmental correlations with both SFA and PUFA (Table 5).

No significant correlations between olive paste traits and olive oil quality indexes, individual fatty acids, or fatty acids categories were found. However, when considering correlations between olive oil quality traits and fatty acids, we found a significant negative phenotypic correlation between K232 on one hand and C18:1 and MUFA on the other, and also a positive phenotypic correlation between K232 on one hand and C18:2 and PUFA on the other.

Regarding the correlations between individual fatty acids and fatty acids categories, we found that SFA presented significant positive phenotypic and environmental correlations with C16:0, positive phenotypic correlations with C16:1, and negative phenotypic and environmental correlations with C18:1 and C20:0. MUFA presented positive phenotypic and environmental correlations with C18:1 and negative phenotypic and environmental correlations with C16:0 and C18:2. Finally, PUFA presented significant negative phenotypic and environmental correlations with C18:1 and positive phenotypic and environmental correlations with C18:2.

3.4. Principal components analysis

The first and second components of the PCA accounted, respectively, for 31.1% and 18.6% of the total variation among variety means (Figure 1). The first component was positively correlated with PUFA and the poliunsaturated fatty acids C18:2 and C18:3, with the total SFA and the short chain saturated fatty acids, including the predominant C16:0 and the minor fatty acid C14:0, and with high values for K232, K270, and peroxide index. This first component was negatively correlated with MUFA and the major monounsaturated fatty acid C18:1 (Figure 1). The second component was positively correlated with total phenolics and K225 parameter, and also with K270 and C20:1. This second component was negatively correlated with C16:1, SFA and the major saturated fatty acid C16:0, as well as with other saturated fatty acids, like C18:0, C20:0, C22:0, and C24:0 (Figure 1).

The projection of the varieties of a two-dimensional PCA plot shows that all the accessions, with the exception of variety Datilera de Caudiel (Dat) and Rogeta de Gorga (Rog) plot in the part of the PCA graph determined by values comprised between -5 and 5 for both the first and second principal components (Figure 2). Variety Datilera de Caudiel (Dat) presents very low values for the second component, while Rogeta de Gorga (Rog) presents very high values for the first component. Datilera de Caudiel

(Dat) ranks first for C16:0, C20:0, C22:0, C24:0, SFA, and C16:1 (Table 4), all of which are traits correlated positively with the second component. Datilera de Caudiel (Dat) also ranks penultimate in fat content, oil yield, and total phenolics, which are negatively correlated with the second component. Regarding Rogeta de Gorga (Rog), for traits positively correlated with the first component, it ranks first for K232 (Table 3) and C18.3 (Table 4), second for C16:0, and third for K270, C18:2, and PUFA; for traits negatively correlated with the first component it ranks last for C18:1 and MUFA (Table 4). The distribution of varieties in the PCA plot according to the category (National, Principal, Secondary, Disseminate, Local, and Minor) shows that varieties belonging to each of the categories are intermingled. However, the less important varieties (Disseminate, Local, and Minor) show a wider area of dispersion in the PCA graph than the commercially important varieties (National, Principal, and Secondary). In this respect, the varieties with highest and lowest values for both the first and second PCA components are always Disseminate, Local, or Minor varieties. Also, most of the National, Principal and Secondary varieties present negative values for the second component. Some of the commercially less important varieties plot very close to commercially important varieties, showing that they present similar characteristics. The comparison of the profiles of varieties that plot close in the PCA analysis using the data for each individual variety shows that they are very similar in olive oil quality indexes, and in fatty acids composition. For example, the Minor variety Calles (Cae) which plots very close to the National varieties Manzanilla Cacereña (MCc) and Hojiblanca (Hoj) is very similar in most traits to the former varieties.

3.5. Discriminant analysis

The discriminant analysis of the 25 traits studied allowed the correct classification according to variety of 179 out of 180 (99.4%) of the samples corresponding to 45 varieties. The only sample which was not correctly classified was the sample of Tempranilla de Ayora (TeA) from the 2006/07 season, which was classified as Genovesa (Gen). This particular sample of Tempranilla de Ayora (TeA) displayed a composition profile more similar to the average of Genovesa (Gen) than to the average of its own variety. The forward stepwise discriminant analysis showed that the same result regarding the correct classification of samples could be achieved using 9 variables: K225, C20:0, C22:0, C16:1, C17:1, C18:1, C20:1, C18:2, C18:3 (Table 6).

The nine discriminant functions obtained were highly significant (P<0.001). The three first discriminant functions explain 80.18% of the variance. For the first discriminant function, the standardized canonical coefficients with greater absolute values correspond to C18:2 (-0.649) and C18:1 (0.630). For the second discriminant function the most relevant standardized canonical coefficients correspond to C22:0 (-0.772), C16:1 (-0.715), C18:3 (0.639) and C18:1 (0.544), and for the third the highest absolute value, with much difference with respect to the others, corresponds to C17:1 (0.876).

4. Discussion

Results revealed that, as occurred for collections from other Mediterranean regions (Barranco & Rallo, 2000; Vinha et al., 2005; Hannachi et al., 2008; Dıraman, 2010; Alba, Bisignano, Rotundo, & Alba, 2012), a great diversity for olive oil traits exist among varieties from the region of Valencia. In this respect, for all traits studied we have detected significant differences among varieties. The ranges of variation for most traits have been very wide, reaching differences of more than 10-fold for total phenolics, a trait which is particularly variable among varieties (Vinha et al., 2005). These results indicate that, even in a single region, a high diversity can be found. Our study also shows that, when compared to some collections from other regions (Rotondi et al., 2011; Alba, Bisignano, Rotundo, & Alba, 2012), a high diversity is found in the region of Valencia, suggesting that accessions from this region represent an important genetic resource for olive selection and breeding.

In general, values obtained for the quality indexes and fatty acids content fall within the ranges of the characteristics established for olive oil composition (The Commission of the European Communities, 1991, 2011). A few exceptions were detected in specific traits were found in some varieties. Although for these varieties the values obtained indicate that they do not comply with the requested standards of quality, in the case of Valentins (Val), the very high content in oleic acid, similar to the highest values obtained in selections among large progenies after directed crosses (León, de la Rosa, Gracia, Barranco, & Rallo, 2008), could be of interest for the good properties of oleic acid on oil stability and human health (Pérez-Jiménez, Ruano, Perez-Martinez, Lopez-Segura, & Lopez-Miranda, 2007; Casal, Malheiro, Sendas, Oliveira, & Pereira, 2010). Also, the very low value of C18:2 of Gileta (Gil) could be of interest for increasing olive oil stability (Frankel, 1985; Casal, Malheiro, Sendas, Oliveira, &

Pereira, 2010). For several traits, like acidity, peroxide index, or K270 and K232 parameters, for which no varieties were found presenting values outside the ranges established by the European legislation (The Commission of the European Communities, 1991, 2011), the results on diversity are of interest, as they are of great relevance for breeding.

One important result is that we have confirmed that although there are differences among seasons in the olive oil characteristics, the season effect is much lower than that of variety (León, Martin, & Rallo, 2004a). This has important implications for chemometry (Stefanoudaki, Kotsifaki, & Koutsaftakis, 2000), as well as for the selection among already existing varieties and for breeding new ones (Rallo, Barranco, de la Rosa, & León, 2008; León et al., 2011). In particular, the season effect has been very low for fatty acids composition, which indicates that fatty acids profile can be used as a fingerprint for identification of monovarietal olive oils (Aparicio & Luna, 2002; Bianchi, di Vincenzo, & Giansante, 2002). The low season effect on fatty acids composition is also relevant for the genetic improvement of the fatty acids profile (León, Martín, & Rallo, 2004a). This is evidenced by the fact that values for the coefficient of genetic variation (CV_G) are close to those of the coefficient of phenotypic variation (CV_P), as well as by high values for heritability (H^2), indicating that selection will be highly efficient (Wricke & Weber, 1986).

When considering the different types of varieties, we found that varieties of the Disseminate and Minor categories are more variable than the commercially important varieties and could represent sources of variation for the improvement of traits of interest. This shows that the ample diversity available in traditional varieties (Bartolini, Prevost, Messeri, & Carignani, 1998) is a genetic resource of great relevance for the breeding of modern varieties adapted to the new requests of the market and demands of the consumers (Fabbri, Lambardi, & Ozden-Tokatli, 2009).

The significant correlations obtained are mostly in agreement with other works (Stefanoudaki, Kotsifaki, & Koutsaftakis, 1999; León, Uceda, Jiménez, Martín, & Rallo, 2004; León, Martín, & Rallo, 2004b; León et al., 2011; Dabbou et al., 2012). The values obtained by us confirm that C18:1 increases stability, while C18:2 decreases it (Frankel, 1985; Ceci & Carelli, 2010). Amazingly, the monounsaturated acid C16:1 is positively correlated with SFA; however, this minor C16:1 fatty acid is correlated positively with C16:0, which is the major saturated acid, and this is the cause of this unexpected correlation (Stefanoudaki, Kotsifaki, & Koutsaftakis, 1999; León, Martín, &

Rallo, 2004b; Dabbou et al., 2012). Also, the positive correlation between phenolics and K225 shows that phenolics are mostly responsible of the bitterness of olive oil (Gutiérrez, Perdiguero, Gutiérrez, & Olías, 1992). We have found that for most significant phenotypic correlations, environmental correlations have also been significant and had the same sign. This suggests that environment may have a relevant role in the observed phenotypic correlations (Wricke & Weber, 1986). On the contrary, for the traits for which the phenotypic correlations are significant, but the environmental correlations are non-significant indicates that a common genetic control may be the reason underlying the phenotypic correlations.

The results obtained by us for the relationships of traits in the PCA plot are in agreement with other works in which PCA analysis have been performed on olive oil composition (León, de la Rosa, Gracia, Barranco, & Rallo, 2008; León et al., 2011). Results from the first component are a clear confirmation of the fact that MUFA, and oleic acid in particular is associated to high stability, while the reverse is true for high PUFA contents (Frankel, 1985). On another hand the second component results indicate that varieties with low content of saturated acids present high contents in phenolics. This is an interesting issue, as low saturated fatty acids content and high content in phenolics is desired in modern varieties (Fabbri, Lambardi, & Ozden-Tokatli, 2009). The multivariate PCA analysis shows that varieties of all categories are spread over a large area in the PCA graph. However, it also reveals that the varieties with less economic importance (Local, Disseminate, and Minor) are spread over a wider area of the PCA graph, suggesting that a wide diversity, of interest for breeding programmes (Fabbri, Lambardi, & Ozden-Tokatli, 2009), is present in these varieties. Also, the PCA allows the identification of varieties with similar and dissimilar characteristics, which may be an important issue in plant breeding.

Some varieties with a very contrasting profile, like Rogeta (Rog) vs. Valentins (Val), or Datliera de Caudiel (Dat) vs. Queixal de Porc (Que), may be of interest for inheritance studies of traits of interest (León, Uceda, Jiménez, Martín, & Rallo, 2004). Also, varieties with a very similar and desired profile may be of interest for intercrossing in order to select individuals in the offspring with a similar or improved composition and better productive characteristics (Fabbri, Lambardi, & Ozden-Tokatli, 2009; Dabbou et al., 2012).

The discriminant analysis shows that it is possible to differentiate varieties and assign correctly the olive oil samples to their variety based on composition traits. In

fact, we have been able to correctly assign all but one of all the olive oil samples. The same result has been obtained when using nine selected traits, of which eight correspond to fatty acids content. Other authors have also found, although with a lower number of varieties (Luna, Morales, & Aparicio, 2006; Lerma-García, Herrero-Martínez, Ramis-Ramos, & Simó-Alfonso, 2008), that discriminant analysis using compositional data is a powerful tool to identify samples of olive oils. This shows again that each variety possesses a characteristic composition fingerprint, in particular a typical fatty acids profile. The other traits considered (acidity, peroxide index, K232, and K270) are highly influenced by the season and residual variation and they are also highly dependent on the conservation status and extraction processes, and therefore are not appropriate for the characterization of varieties (Vekiari, Papadopoulou, & Kiritsakis, 2007). This information is very relevant for the application of chemometry for the identification of samples, as well as to detect fraud, and may be an indication that composition traits, in particular fatty acids, can be as precise for varietal identification as molecular markers (Stefanoudaki, Kotsifaki, & Koutsaftakis, 2000; Belaj, Trujillo, de la Rosa, Rallo, & Giménez, 2001).

In summary, our results show that a wide diversity exists within the Collection of Olive Varieties of the Region of Valencia. In particular, a greater diversity exists in the economically less important varieties, indicating that selection among them for specific composition profiles with desirable properties (i.e., high K232 and K270 values, high oleic acid content, low content saturated fatty acids, etc.) can be of interest for the recovery of neglected varieties. The fact that heritability values for fatty acid contents is very high indicates that selection for these traits will be highly efficient and, together with discriminant analysis results, confirms that fatty acids profile is a powerful tool for chemometry and chemical fingerprinting of olive oil varieties. The results obtained also provide relevant information for breeding programmes aimed at developing new olive oil varieties through directed crosses.

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Table 1

Olive varieties and type of variety used in the analysis of olive paste, olive oil quality indexes, and fatty acids composition in a collection from the region of Valencia.

Variety	Code	Category[a]
Aguilar	Agu	E
Alfafara	Alf	B
Arbequina	Arb	A
Blanqueta	Bla	B
Blanqueta Gorda	BlG	D
Borriolenca	Bor	C
Cabaret	Cab	E
Calles	Cae	F
Callosina	Cao	C
Carrasqueta de Ayora	CaA	E
Carrasqueña de la Cañada	CaC	E
Changlot Real	ChR	B
Changlotera de Liria	ChL	F
Cornicabra	Cor	A
Cuquellos	Cuq	F
Datilera de Caudiel	Dat	F
Dulce de Ayora	Dul	E
Empeltre	Emp	A
Fraga	Frg	F
Frantoio	Frn	A
Genovesa	Gen	D
Gileta	Gil	F
Gorda Limoncillo	Gor	E
Hojiblanca	Hoj	A
Lloma	Llo	E
Manzanilla Cacereña	MCc	A
Manzanilla de Caudiel	MCu	E
Matias	Mat	F
Morona de Castellón	MoC	E
Morruda de Salinas	MoS	E
Otos	Oto	F
Picual	Pia	A
Picudo	Pid	A
Queixal de Porc	Que	F
Racimo	Rac	E
Rogeta de Gorga	Rog	F
Rojal de Valencia	Roj	C
Serrana de Espadán	Ser	B
Sollana	Sol	C
Temprana de Montán	TeM	D
Tempranilla de Ayora	TeA	F
Tio Blas	Tio	E
Valentins	Val	E
Vera de Valencia	Ver	E
Villalonga	Vil	B

[a]Category according to the economic importance of the variety: A=National, B=Principal; C=Secondary; D=Disseminate, E=Local, F=Minor.

2 Mean, percentage of the total sum of squares and significance for the effects of season,

3 variety, and residual, coefficient of phenotypic variation (CV_P), coefficient of genotypic

4 variation (CV_G), and broad-sense heritability (H^2) for the olive paste and olive oil traits

5 in a collection of 45 varieties from the region of Valencia.

Trait	Sums of squares (%)			Mean	CV_P (%)	CV_G (%)	H^2
	Season[a]	Variety[a]	Residual				
Olive paste traits							
Moisture (%)	13.60***	37.54***	48.86	51.5	13.99	6.94	0.25
Fat content (%)	9.62***	50.69***	39.68	22.5	23.38	15.05	0.41
Oil yield (%)	14.54***	48.23***	37.24	17.6	32.74	21.19	0.42
Olive oil quality indexes							
Acidity (%)	2.55[ns]	32.31*	65.14	0.40	69.78	23.01	0.11
Peroxide index (mEq $O_2 \cdot kg^{-1}$)	8.41***	46.13***	45.46	5.51	56.72	32.99	0.34
K232	2.69*	54.49***	42.82	1.62	18.37	11.81	0.41
K270	18.21***	44.57***	37.22	0.12	33.90	21.26	0.39
Total phenolics (mg·kg^{-1})	7.26***	61.54***	31.20	243	71.04	52.75	0.55
K225	3.46**	66.47***	30.07	0.18	73.56	56.25	0.58
Individual fatty acids							
C14:0	3.09**	65.19***	31.73	0.01	44.10	33.10	0.56
C16:0	3.10***	89.19***	7.72	12.47	23.32	22.05	0.89
C17:0	2.88***	82.45***	14.67	0.09	73.25	65.46	0.80
C18:0	6.74***	76.47***	16.79	2.34	23.59	20.57	0.76
C20:0	5.13***	75.02***	19.84	0.38	15.07	12.79	0.72
C22:0	1.64*	77.47***	20.89	0.12	18.37	15.55	0.72
C24:0	4.36**	58.05***	37.60	0.06	20.28	13.99	0.48
C16:1	0.17[ns]	92.53***	7.31	1.25	61.68	58.60	0.90
C17:1	0.60[ns]	89.14***	10.26	0.18	65.36	60.69	0.86
C18:1	0.85***	96.78***	2.38	70.94	10.31	10.14	0.97
C20:1	0.79*	88.34***	10.87	0.32	26.41	24.40	0.85
C18:2	0.24**	97.33***	2.42	11.10	49.89	49.08	0.97
C18:3	1.33*	78.59***	20.09	0.75	23.13	19.75	0.73
Fatty acid categories							
SFA	4.22***	87.72***	8.06	15.47	19.50	18.37	0.89
MUFA	0.92***	96.67***	2.41	72.69	9.62	9.46	0.97
PUFA	0.23**	97.32***	2.45	11.84	47.38	46.60	0.97

6 [a] ***, **, *, [ns] indicate, respectively, significant at P<0.001, <0.01, <0.05, and non-

7 significant.

Table 3

Mean values and standard deviation for each category, average, minimum and maximum values, and standard error (SE) for individual variety means for the olive paste traits and olive oil quality indexes in the olive varieties studied. For minimum and maximum values, the code of the variety and category are indicated between brackets.

Traits	National (A)	Principal (B)	Secondary (C)	Disseminate (D)	Local (E)	Minor (F)	Average	Minimum	Maximum	SE
				Category						
n	8	5	4	3	14	11	45			
Oil paste traits										
Moisture (%)	47.9±6.1	47.7±3.7	49.3±3.4	51.8±2.5	53.1±3.1	54.5±4.7	51.5	37.9 (Frn-A)	62.0 (Mat-F)	3.2
Fat content (%)	24.3±4.2	24.9±3.6	23.4±2.4	22.9±2.8	21.9±3.2	20.3±4.3	22.5	14.0 (Mat-F)	30.8 (Frn-A)	2.0
Oil yield (%)	17.3±4.5	19.1±3.9	17.3±1.1	16.7±2.2	15.4±2.8	14.2±4.4	16.1	7.6 (Mat-F)	23.8 (Vil-B)	2.1
Olive oil quality traits										
Acidity (%)	0.36±0.17	0.34±0.13	0.34±0.12	0.35±0.05	0.48±0.18	0.37±0.18	0.40	0.17 (Agu-E)	0.83 (Rog-F)	0.14
PI (mEq $O_2 \cdot kg^{-1}$)[a]	5.3±2.0	5.7±1.8	4.9±2.1	4.9±0.9	5.4±2.2	6.0±3.7	5.5	1.5 (Dul-E)	13.0 (Cuq-F)	1.4
K270	0.11±0.02	0.13±0.02	0.12±0.06	0.14±0.02	0.11±0.02	0.13±0.03	0.12	0.08 (Bor-C)	0.21 (Sol-C)	0.01
K232	1.53±0.17	1.70±0.21	1.60±0.28	1.79±0.29	1.56±0.19	1.70±0.23	1.62	1.17 (Dul-E)	2.21 (Rog-F)	0.11
TP ($mg \cdot kg^{-1}$)[a]	248±92	298±54	261±258	302±141	181±68	273±176	243	55 (CaA-E)	646 (Sol-C)	54
K225	0.17±0.07	0.25±0.06	0.19±0.22	0.25±0.13	0.14±0.06	0.19±0.14	0.18	0.05 (Bor-C)	0.52 (Sol-C)	0.04

[a]PI=Peroxide index; TP=Total phenolics.

Table 4

Mean values and standard deviation for each category, average, minimum and maximum values, and standard error (SE) for individual variety means for the fatty acids content (in percentage) in the olive oil of the olive varieties studied. For minimum and maximum values, the code of the variety and category are indicated between brackets.

Fatty acid (%)	Category						Average	Minimum	Maximum	SE
	National (A)	Principal (B)	Secondary (C)	Disseminate (D)	Local (E)	Minor (F)				
n	8	5	4	3	14	11	45			
Saturated										
C14:0	0.011±0.003	0.015±0.004	0.014±0.004	0.017±0.007	0.012±0.003	0.015±0.008	0.013	0.010 (Agu-E)	0.030 (Gil-F)	0.002
C16:0	12.1±2.3	12.9±1.3	13.5±2.4	14.0±3.6	11.7±2.9	12.8±3.7	12.5	7.4 (Llo-E)	20.4 (Dat-F)	0.5
C17:0	0.08±0.05	0.18±0.05	0.07±0.06	0.08±0.06	0.09±0.05	0.08±0.05	0.09	0.03 (CaC-E)	0.26 (Ser-B)	0.01
C18:0	2.54±0.81	2.24±0.15	2.50±0.19	2.42±0.66	2.35±0.43	2.14±0.38	2.34	1.50 (Emp-A)	3.53 (Pia-A)	0.13
C20:0	0.39±0.06	0.40±0.04	0.39±0.06	0.40±0.06	0.38±0.05	0.37±0.05	0.38	0.31 (Cao-C)	0.52 (Dat-F)	0.02
C22:0	0.11±0.01	0.13±0.02	0.11±0.01	0.11±0.01	0.11±0.02	0.12±0.03	0.12	0.08 (MoC-E)	0.18 (Dat-F)	0.01
C24:0	0.05±0.01	0.06±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.06	0.04 (Cao-C)	0.08 (Dat-F)	0.004
Total SFA	15.2±1.8	15.9±1.2	16.7±2.4	17.1±4.0	14.7±3.2	15.6±3.7	15.5	10.5 (Llo-E)	23.7 (Dat-F)	0.51
Monounsaturated										
C16:1	1.12±0.47	1.07±0.41	1.44±0.75	0.98±0.43	1.20±0.60	1.49±1.22	1.25	0.43 (Llo-E)	4.78 (Dat-F)	0.12
C17:1	0.14±0.08	0.35±0.03	0.14±0.10	0.14±0.11	0.18±0.10	0.16±0.11	0.18	0.06 (Que-F)	0.42 (Agu-E)	0.02
C18:1	73.6±6.1	67.2±5.7	69.9±6.7	64.5±8.5	74.4±6.3	68.5±7.9	70.9	56.3 (Rog-F)	84.3 (Val-E)	0.65
C20:1	0.30±0.04	0.32±0.07	0.29±0.06	0.35±0.15	0.31±0.09	0.35±0.09	0.32	0.21 (MCu-E)	0.55 (Gil-F)	0.02
Total MUFA	75.2±5.7	69.0±5.6	71.7±6.3	66.0±8.5	76.0±6.1	70.5±7.3	72.7	58.8 (Rog-F)	85.6 (Val-E)	0.63
Poliunsaturated										
C18:2	8.9±4.2	14.4±4.5	10.9±5.2	16.2±6.1	8.6±4.4	13.1±5.9	11.1	3.3 (Agu-E)	22.9 (Gil-F)	0.49
C18:3	0.73±0.14	0.74±0.13	0.71±0.24	0.78±0.18	0.71±0.14	0.82±0.16	0.75	0.44 (Bor-C)	1.19 (Rog-F)	0.05
Total PUFA	9.6±4.3	15.1±4.5	11.6±5.4	17.0±6.3	9.3±4.5	13.9±6.0	11.8	3.9 (Agu-E)	23.7 (Gil-F)	0.50

Table 5

19 Values of the phenotypic and environmental correlation coefficient for pairs of traits for

20 which the phenotypic correlation has been significant according to the Bonferroni test

21 (P≤0.05).

Trait 1	Trait 2	Phenotypic correlation	Environmental correlation[a]
Among olive paste traits			
Moisture	Fat content	-0.882	-0.915
Moisture	Oil yield	-0.875	-0.917
Fat content	Oil yield	0.953	0.913
Among olive oil quality indexes			
K232	K270	0.686	0.425
K270	Total phenolics	0.735	0.169[ns]
K270	K225	0.753	0.316
Total phenolics	K225	0.911	0.681
Among individual fatty acids			
C16:0	C16:1	0.811	-0.011[ns]
C16:0	C18:1	-0.745	-0.664
C16:0	C20:1	-0.625	-0.359
C17:0	C17:1	0.935	0.511
C18:0	C20:0	0.653	0.555
C20:0	C22:0	0.754	0.620
C22:0	C24:0	0.633	0.531
C18:1	C18:2	-0.887	-0.563
Among fatty acids categories			
SFA	MUFA	-0.660	-0.722
MUFA	PUFA	-0.915	-0.547
Olive oil traits - Individual fatty acids			
K232	C18:1	-0.676	-0.072[ns]
K232	C18:2	0.734	0.182[ns]
Olive oil traits - Fatty acids categories			
K232	MUFA	-0.690	-0.050[ns]
K232	PUFA	0.739	0.190[ns]
Individual fatty acids – Fatty acids categories			
C16:0	SFA	0.984	0.962
C16:0	MUFA	-0.698	-0.691
C16:1	SFA	0.786	-0.073[ns]
C18:1	SFA	-0.706	-0.685
C18:1	MUFA	0.996	0.987
C18:1	PUFA	-0.886	-0.573
C20:1	SFA	-0.651	-0.394
C18:2	MUFA	-0.916	-0.542
C18:2	PUFA	0.999	0.996

22 [a]Environmental correlation values are significant at P≤0.05 according to the Bonferroni

23 test, except when [ns] is indicated, in which case they are non-significant.

24

25 **Table 6**

26 Standarized canonical coefficients for the nine discriminant functions obtained after

27 applying a forward stepwise procedure for the classification of oil samples according to

28 variety. Coefficient values greater than 0.5 are represented in bold. All discriminant

29 functions were highly significant (P<0.001).

|	Functions								
	1	2	3	4	5	6	7	8	9
K225	-0.318	0.247	-0.223	0.136	-0.162	-0.423	**-0.741**	0.158	0.472
C18:0	0.328	0.081	-0.004	**0.592**	**0.637**	**0.689**	-0.168	0.441	-0.063
C22:0	-0.076	**-0.772**	0.062	-0.206	**0.564**	**-0.759**	0.335	0.315	-0.046
C16:1	0.379	**-0.715**	0.208	0.339	-0.240	0.394	0.080	0.375	**0.528**
C17:1	-0.102	0.116	**0.876**	-0.408	-0.169	0.220	-0.217	0.190	0.001
C18:1	**0.630**	**0.544**	-0.007	-0.240	0.440	0.237	0.490	**0.576**	**0.795**
C20:1	-0.297	0.469	0.460	**1.038**	-0.425	0.129	-0.112	0.007	-0.194
C18:2	**-0.649**	0.278	-0.014	-0.063	**0.544**	0.246	0.497	0.374	**0.629**
C18:3	-0.080	**0.639**	-0.622	-0.277	-0.496	-0.029	-0.172	**0.573**	-0.318
Eigenvalue	53.97	30.66	11.42	5.84	4.50	2.33	1.56	1.06	0.94
Variance (%)	48.07	27.31	10.17	5.20	4.01	2.07	1.39	0.94	0.84
Canonical correlation	0.991	0.984	0.959	0.924	0.905	0.836	0.780	0.717	0.697

30
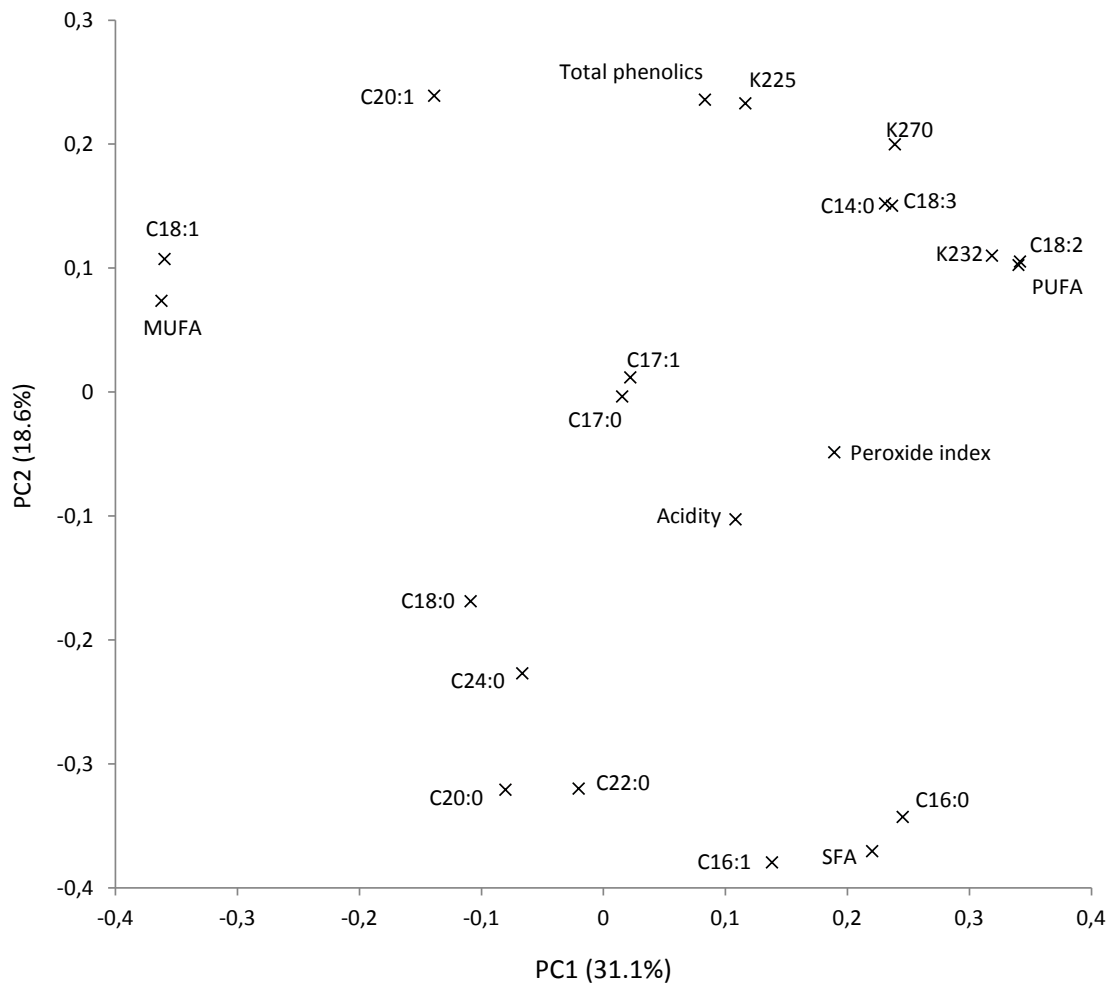
Fig. 1. Diagram showing the relationships among the 22 olive oil traits considered based on the two first principal components of PCA (31.1% and 18.6% of the total variation, respectively). Results are based on the data obtained from mean values of 45 olive varieties from the region of Valencia (Spain).
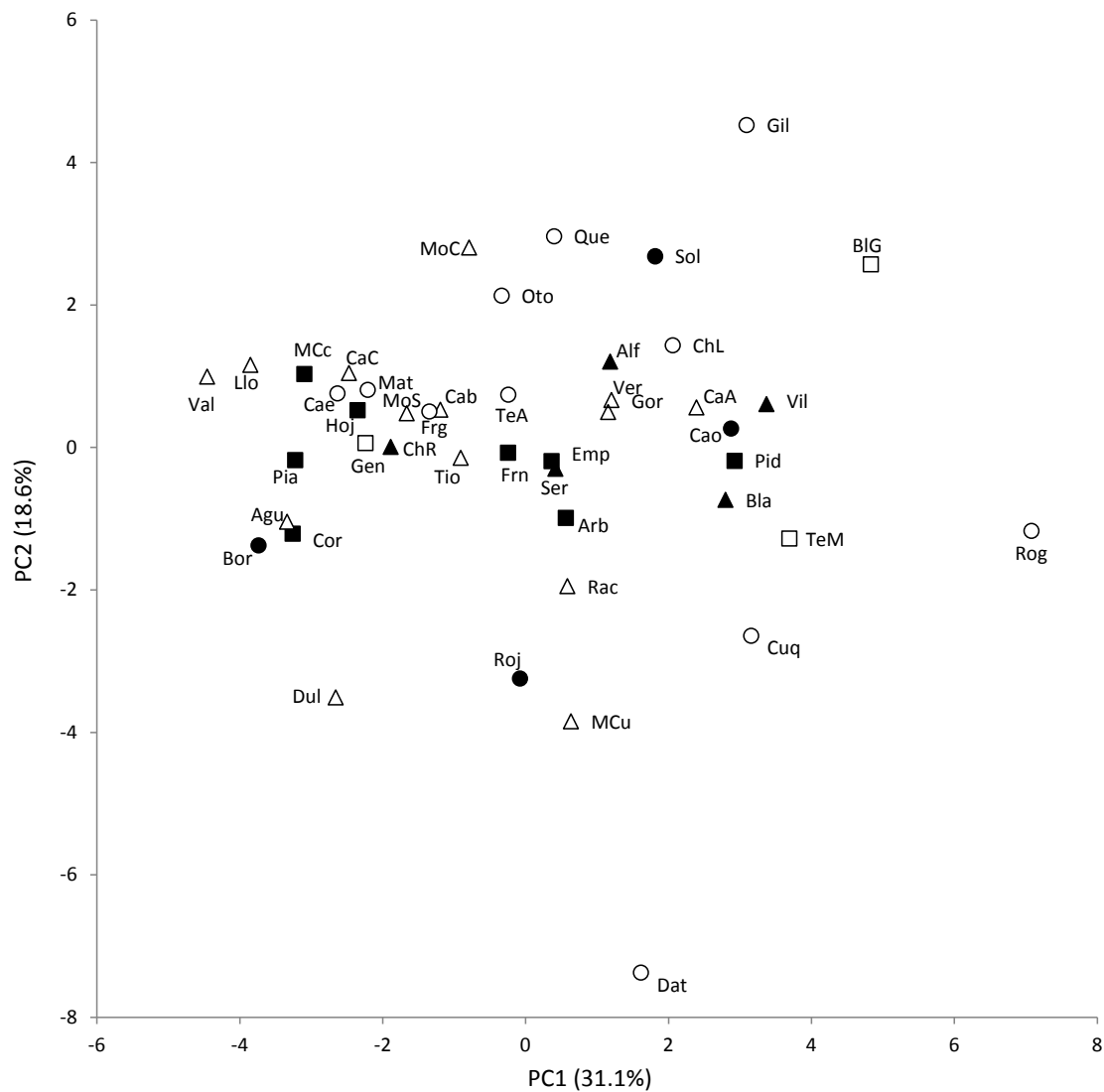
Fig. 2. Similarities based on 22 olive oil traits among 45 accessions of 45 olive varieties from the region of Valencia (Spain) represented on the two first principal components of PCA (31.1% and 18.6% of the total variation, respectively). The different cultivar categories (according to the economic importance of the variety) are represented by different symbols: National (filled square), Principal (filled triangle), Secondary (filled circle), Disseminate (open square), Local (open triangle), Minor (open circle).