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ARTICLE

# Triterpenic Content and Chemometric Analysis of Virgin Olive Oils from Forty Olive Cultivars

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Forty olive cultivars (*Olea europaea*, L.) from the World Olive Germoplasm Bank Collection of Cordoba (Spain) were studied for their oil triterpenic dialcohol (uvaol and erythrodiol) and acid (oleanolic, ursolic, maslinic) composition. Dialcohol content ranged from 8.15 to 85.05 mg/kg, erythrodiol being the most predominant (from 5.89 to 73.78 mg/kg), whereas uvaol content was found at lower levels (from 1.50 to 19.35 mg/kg). Triterpenic acid concentration oscillated between 8.90 to 112.36 mg/kg. Among them, ursolic acid was found at trace levels, while the mean values of oleanolic and maslinic acids ranged from 3.39 to 78.83 mg/kg and 3.93 to 49.81 mg/kg, respectively. The variability observed for both triterpenic dialcohols and acid content was emphasized by principal component and cluster analyses. Both analyses were able to discriminate between oil samples, especially by erythrodiol, oleanolic acid, and maslinic acids. Regarding these results, we conclude that the virgin olive oil triterpenic fraction can be considered as a useful tool to characterize monovarietal virgin olive oil.

**KEYWORDS:** ***Olea europaea*** **L.; cultivars; virgin olive oil; triterpenic dialcohols; triterpenic acids; chemometrics**

## INTRODUCTION

The olive tree (*Olea europaea*, L.) has been widely cultivated in the Mediterranean countries from antiquity. In these areas, extra virgin olive oil, the major source of dietary fat, constitutes part of the commonly called Mediterranean diet, the consumption of which has been associated with a low incidence of cardiovascular diseases (*1*−*3*), cancer (*4*−*6*), and oxidative stress (*7*, *8*). These health benefits have long been attributed to its unique composition: a high content of monounsaturated fatty acids (oleic acid) and minor compounds such as tocopherols, polyphenols, triterpenoids, and squalene. Nevertheless, the level of these compounds in virgin olive oils can be influenced by a great number of factors; among them, the cultivar has been largely reported (*9*−*11*). In order to evaluate the importance of the olive cultivar and its intraspecific variability, the World Olive Germoplasm Bank Collection of Cordoba (more than 358 olive cultivars) is being studied from different aspects such as agronomic, oil composition, and sensory characteristics. Oils studies have shown that the genetic factor is the most important component in the variability for fatty acids, polyphenols, tocopherols (*12*), and sensory properties (*13*). The influence of the olive cultivar on oil composition has also been described by other authors (*14*−*16*) for other olive collections.

Among the minor compounds present in the unsaponifiable fraction of virgin olive oil can be found the pentacyclic triterpenes: oleanolic acid, ursolic acid, maslinic acid, uvaol, and erythrodiol. Several studies have shown that these compounds possess healthy properties such as anti-inflammatory (*17*, *18*), vasodilatory (*19*, *20*), antioxidant (*21*−*23*), and antitumoral (*24*, *25*) properties. However, there is no data about their concentration in virgin olive oil since triterpenic dialcohols (uvaol and erythrodiol) are used as a purity parameter to detect pomace olive oil (*26*) and are expressed as the sum of percent of total sterols; whereas for acids (oleanolic, ursolic, and maslinic), there is one work describing the analytical method as well as the effect of several technological factors on their content in olive oil (*27*). In addition, works dealing with triterpenic acids generally referred to their presence in crude pomace olive oil because of their high content (mean content 2690 mg/kg) (*27*), and virgin olive oil is neglected. According to EU regulations (*26*), crude pomace oil cannot be consumed directly and needs to be refined, thus removing all of the triterpenic acids. Therefore, these data indicate that virgin olive oil can be considered a unique source of these compounds. Because of the lack of data on triterpenic compound concentration in virgin olive oil, the aim of the present work was to perform a screening on 40 cultivars, from the World Olive Germoplasm Bank Collection of Cordoba, to describe the triterpene concentration of their virgin olive oils. The obtained results were then subjected to multivariate analysis in order to evaluate these compounds as a tool for cultivar discrimination.

## MATERIAL AND METHODS

**Plant Material.** The study was carried out on 40 olive cultivars from the World Olive Germoplasm Bank Collection of Cordoba (Spain).

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**Table 1.** Area of Origin of the 40 Olive Cultivars Grown in the World Olive Germoplasm Bank Collection of Cordoba, Spain

| cultivar | origin |
|---|---|
| Arbequina | Spain |
| Blanqueta | Spain |
| Cakir o Valanolia | Greece−Turkey |
| Callosina | Spain |
| Changlot real | Spain |
| Chetoui | Tunisia |
| Cipresino | Italy |
| Cobrancosa | Portugal |
| Cordovil de serpa | Portugal |
| Cornicabra | Spain |
| Crnica | Egypt |
| Dolce Agogia | Italy |
| Empeltre | Spain |
| Frantoio | Italy |
| Galega Vulgar | Portugal |
| Genovesa | Spain |
| Hojiblanca | Spain |
| Kel-Et Ter 145 | Syrie |
| Lechin de Granada | Spain |
| Lechin de Sevilla | Spain |
| Loaime | Spain |
| Manzanilla Cacerea | Spain |
| Manzanilla de Sevilla | Spain |
| Megaritiki | Greece |
| Moraiolo | Italy |
| Nevado Azul | Spain |
| Pajarero | Spain |
| Picholine Marrocaine | Morroco |
| Pico Limon de Grazalema | Spain |
| Picual | Spain |
| Picudo | Spain |
| Racimal | Spain |
| Royal de Calatayud | Spain |
| Salonenque | France |
| Sevillenca-1 | Spain |
| St. George Greys | USA |
| Tempranillo de Calatayud-CJ | Spain |
| Verdial De Badajoz | Spain |
| Zalmati | Tunisia |
| Zarza | Spain |

The areas of origin of these cultivars are shown in **Table 1**. For each cultivar, two trees were selected on the basis of uniformity and yield index (between 3 and 4). The olive trees were spaced 7 × 7 m and grown using traditional practices.

**Fruit Sampling.** For each cultivar, one sample (5 kg) per tree was harvested when the most abundant ripening stage in the tree was 3, according to fruit classification based on skin and flesh color described in the ripening index method (*28*).

**Oil Extraction.** Oil extraction was performed using an Abencor laboratory oil mill (Abengoa, Seville). The fruits were crushed in a hammer mill, and the olive paste was kneaded for 30 min at 28 °C and then centrifuged for 1 min at 3500 rpm. The oily must was left for decantation and then filtered. Oils were stored at −20 °C until analysis.

**Determination of Triterpenic Dialcohols.** The analysis of uvaol and erythrodiol was performed according to EU Regulation 2568/91 (*26*) for the determination of sterols in olive oil. The oil sample was saponified with ethanolic potassium hydroxide solution. The unsaponifiable fraction was removed with ethyl ether, and the sterol fraction was separated by Silicagel plate chromatography. Separation and quantification of silylated sterol was performed on a Helwett Packard instrument model 6890 gas chromatograph, equipped with a HP-5 capillary column (25 m, 0,25 mm i.d., 0,25 μm of thickness). The working conditions were oven temperature, 260 °C; injector at 305 °C split/spliteless; FID detector, 330 °C. The injected volume was 1 μL at a flow rate 1 mL/min, using helium as carrier gas. For quantification, betulin was used as the internal standard. The same response factor was considered for both triterpenic dialcohols uvaol and erythrodiol. Analyses were performed in duplicate, and results were expressed as mg/kg.

**Determination of Triterpenic Acids.** The acidic fraction was isolated by solid phase extraction using bonded aminopropyl cartridges, and betulinic acid was added as internal standard according to the method described by Pérez-Camino and Cert (*27*). Then, the extract was evaporated, silylated, and analyzed by gas chromatography. The chromatographic analysis was performed using a Perkin-Elmer gas chromatograph, autosystem model, fitted with a flame ionization detector and a split injection system (split ratio 1: 0.25). Separation was carried out on an HP-5 capillary column (30 m, 0.32 mm i.d, 0.25 μm of thickness).The operating conditions were oven temperature, 260 °C for 5 min and then increased at 4 °C/min up to 320 °C; injector and detector at 320 °C. Helium was used as carrier gas at a column head pressure of 25 psi. The pentacyclic triterpenes were quantified assuming the same response factor for all triterpenic acids. Analyses were performed in duplicate, and results were expressed as mg/kg of betulinic acid.

**Statistical Analysis.** The results for each cultivar are reported as the mean ± standard deviation (SD), while for all cultivars, standard error (S.E) and coefficient of variation are reported. These determinations were carried out using the program Statistix, version 8.0.

Principal component analysis and cluster analysis are multivariate statistical studies and were performed in order to discriminate the 40 monovarietal virgin olive oils according to their triterpenic profile similarity. Both analyses were performed with the program Unscrambler (Unscrambler software, version 9.6).

## RESULTS AND DISCUSSION

**Triterpenic Dialcohols. Table 2** reports the triterpenic dialcohol composition of the 40 virgin olive oils, and values are expressed as mg/kg. Results showed a great variability in the content of both dialcohols obtaining a coefficient of variation higher than 70%. In all genotypes, erythrodiol was the predominant one and accounted for 78% of the total. Concentrations ranged from 5.89 to 73.78 mg/kg for the cultivars Frantoio and Nevado Azul, respectively. Frantoio was the exception since its uvaol content (8.37 mg/kg) was higher than that of erythrodiol (5.89 mg/kg). Uvaol values ranged from 1.50 mg/kg to 19.35 mg/kg, respectively, for the cultivars Genovesa and Dolce Agogia. These data were subjected to principal component and cluster analyses.

The principle of principal component analysis is finding the linear combinations of the initial variables that highly contribute to making the samples different from each other. Each component of a principal component model is characterized by three complementary sets of attributes which are variances that are error measures, loadings describing the data structure in terms of variable correlations, and scores describing the properties, differences, or similarities of the samples. However, the principle of cluster analysis is to group the samples into $k$ numbers of clusters based on certain specific distance measurements. The cluster analysis is repeated with a large number of iterations, and the optimal clustering result was retained when the lowest value of the sum of distances is obtained. In this work, we established 5 groups of clusters considering the Euclidean distances.

Variance results from the principal component analysis showed that erythrodiol explains 96% of the data variance (PC 1), while uvaol contributes only by 4% (PC 2). The visualization of the 40 cultivars (scores) as well as their distribution into groups according to principal component and cluster analyses are reported in **Figure 1**. The olive cultivar groups established were as follows:

Group I: Very high content of erythrodiol.
Group II: High content of erythrodiol.
Group III: Intermediate content of erythrodiol.

**Table 2.** Triterpenic Dialcohol Composition of the 40 Monovarietal Virgin Olive Oils Obtained from Cultivars of the World Olive Germoplasm Bank Collection of Cordoba (Spain)[a]

| cultivar | group | uvaol (mg/kg) | erythrodiol (mg/kg) | % uvaol + erythrodiol | Σ uvaol + erythrodiol (mg/kg) |
|---|---|---|---|---|---|
| Nevado Azul | I | 11.28 ± 6.43 | 73.78 ± 22.06 | 4.05 ± 0.35 | 85.06 ± 15.63 |
| Lechin de Granada | I | 18.36 ± 9.36 | 66.43 ± 8.34 | 4.36 ± 1.05 | 84.78 ± 17.69 |
| Moraiolo | II | 10.42 ± 9.96 | 51.58 ± 31.89 | 3.95 ± 2.62 | 62.00 ± 41.85 |
| Dolce Agogia | II | 19.35 ± 8.12 | 49.35 ± 5.75 | 3.90 ± 0.28 | 68.69 ± 13.87 |
| Cornicabra | II | 17.86 ± 1.90 | 47.33 ± 1.24 | 4.16 ± 0.21 | 65.18 ± 3.14 |
| Solonenque | II | 7.44 ± 5.93 | 43.73 ± 2.34 | 2.40 ± 2.29 | 51.17 ± 8.27 |
| Verdial de Badajoz | II | 5.40 ± 1.70 | 43.40 ± 16.69 | 3.50 ± 1.27 | 48.80 ± 18.39 |
| Sevillenca-1 | III | 5.82 ± 4.48 | 37.36 ± 12.32 | 1.75 ± 0.50 | 43.18 ± 16.80 |
| Picholine Marrocaine | III | 7.83 ± 4.95 | 36.36 ± 8.72 | 2.06 ± 0.49 | 44.19 ± 13.67 |
| Cordovil de Serpa | III | 4.45 ± 3.04 | 31.30 ± 0.71 | 3.20 ± 0.42 | 35.75 ± 3.75 |
| Cipressino | III | 9.88 ± 1.89 | 31.10 ± 9.54 | 2.80 ± 0.42 | 40.97 ± 7.65 |
| Arbequina | III | 9.36 ± 1.06 | 30.67 ± 6.49 | 2.70 ± 0.28 | 40.03 ± 5.43 |
| Callosina | III | 13.35 ± 5.96 | 30.18 ± 12.68 | 2.20 ± 0.85 | 43.52 ± 18.64 |
| Cakir o Valanolia | III | 10.00 ± 4.10 | 29.25 ± 1.20 | 2.20 ± 0.00 | 39.25 ± 2.90 |
| Kelb-Et Ter 145 | III | 5.60 ± 1.10 | 29.18 ± 11.04 | 2.70 ± 0.14 | 34.78 ± 9.93 |
| Royal de Calatayaud | III | 2.66 ± 0.62 | 26.93 ± 2.93 | 1.78 ± 0.04 | 29.59 ± 3.55 |
| Zalmati | IV | 5.78 ± 0.12 | 23.74 ± 10.13 | 1.23 ± 0.39 | 29.52 ± 10.01 |
| Picudo | IV | 5.10 ± 2.83 | 23.55 ± 10.96 | 1.30 ± 0.71 | 28.65 ± 13.79 |
| Hojiblanca | IV | 2.90 ± 0.85 | 23.30 ± 3.94 | 0.70 ± 0.14 | 26.20 ± 4.24 |
| Cobrancosa | IV | 10.45 ± 2.76 | 22.15 ± 3.75 | 1.90 ± 0.42 | 32.60 ± 6.51 |
| Blanqueta | IV | 6.79 ± 1.65 | 21.92 ± 0.92 | 2.22 ± 0.26 | 28.71 ± 2.57 |
| Picual | IV | 9.15 ± 0.35 | 21.35 ± 8.84 | 2.15 ± 0.21 | 30.50 ± 8.49 |
| Racimal | IV | 3.84 ± 1.59 | 21.06 ± 1.67 | 1.45 ± 0.21 | 24.90 ± 0.08 |
| Chetoui | IV | 10.24 ± 3.51 | 20.94 ± 0.48 | 2.50 ± 0.00 | 31.18 ± 4.00 |
| Tempranillo de Calatayaud | IV | 3.75 ± 1.63 | 18.90 ± 1.41 | 1.15 ± 0.07 | 22.65 ± 0.21 |
| Manzanilla de Sevilla | IV | 3.27 ± 1.57 | 18.42 ± 7.59 | 1.63 ± 0.39 | 21.69 ± 9.16 |
| Loaime | IV | 9.00 ± 0.42 | 17.90 ± 0.28 | 1.80 ± 0.14 | 26.90 ± 0.14 |
| Empeltre | IV | 3.95 ± 0.07 | 17.65 ± 2.33 | 1.35 ± 0.21 | 21.60 ± 2.26 |
| Changlot Real | IV | 7.30 ± 2.12 | 16.45 ± 0.64 | 3.05 ± 0.50 | 23.75 ± 1.48 |
| Megaritiki | IV | 6.33 ± 2.87 | 16.40 ± 1.94 | 1.55 ± 0.35 | 22.73 ± 4.81 |
| Zarza | IV | 3.28 ± 2.19 | 15.16 ± 1.17 | 0.98 ± 0.23 | 18.43 ± 3.35 |
| Pajarero | IV | 3.06 ± 0.19 | 14.81 ± 0.16 | 0.86 ± 0.06 | 17.87 ± 0.35 |
| Pico Limon de Grazalema | IV | 4.20 ± 0.85 | 14.55 ± 0.78 | 1.95 ± 0.21 | 18.75 ± 1.63 |
| Galega Vulgar | IV | 8.10 ± 1.42 | 14.54 ± 3.01 | 1.20 ± 0.00 | 22.63 ± 4.43 |
| Lechin de Sevilla | V | 2.20 ± 0.14 | 9.50 ± 2.40 | 0.55 ± 0.21 | 11.70 ± 2.55 |
| Genovesa | V | 1.50 ± 0.42 | 8.25 ± 1.20 | 1.35 ± 0.21 | 9.75 ± 1.63 |
| Crnica | V | 5.91 ± 3.15 | 7.97 ± 4.52 | 1.05 ± 0.35 | 13.87 ± 7.67 |
| St. George Greys | V | 1.70 ± 0.52 | 6.78 ± 2.28 | 0.46 ± 0.06 | 8.48 ± 1.75 |
| Manzanilla Cacerea | V | 1.55 ± 0.21 | 6.60 ± 3.39 | 0.60 ± 0.28 | 8.15 ± 3.61 |
| Frantoio | V | 8.37 ± 6.11 | 5.89 ± 1.72 | 0.67 ± 0.19 | 14.26 ± 7.83 |
| average of 40 cultivars ± SE | | 7.17 ± 0.58 | 26.14 ± 1.88 | 2.03 ± 0.13 | 33.31 ± 2.28 |
| C.V (%) | | 71.96 | 64.29 | 56.89 | 61.22 |

[a] Mean values ± standard deviation; S.E., standard error of the mean; C.V., coefficient of variation.

Group IV: Intermediate to low content of erythrodiol.
Group V: Low content of erythrodiol.

The content of uvaol + erythrodiol expressed as the percentage of total sterols is reported in **Table 2**. This parameter is included as a quality index in EU regulations. In extra virgin olive oil, it must not exceed 4.5% of total sterols (*26*) because higher values indicate blending with olive pomace oil. In our study, the values obtained filled within the limits established by EC Regulation 2568/91 (*26*) for the category Extra Virgin Olive Oil. Exceptions were found for the cultivars Lechin de Granada and Moraiolo that showed values higher than the limits established by the EC Regulation, and thus, new studies focused on these cultivars should be performed.

The sum of erythrodiol and uvaol content expressed as mg/kg is also shown in **Table 2**. For this parameter, a wide variation was observed between the different oil samples, ranging from 8.15 mg/kg in Manzanilla Cacereña oil to 85 mg/kg in Nevado Azul and Lechin de Granada oils.

**Triterpenic Acids.** The mean content of oleanolic, ursolic, and maslinic acids are shown in **Table 3**. As we can observe, ursolic acid is present at trace in most of the monovarietal virgin olive oils, while it was not detected in some of them. Our results are in agreement with those described by others authors for olive fruit (*29*−*32*). Among the cultivars studied, Zalmati virgin olive oil showed the highest content of ursolic acid (4.07 mg/kg). In addition, data of **Table 3** indicate that the mean values of oleanolic and maslinic acids for the pool of cultivars were similar with an average content of 17.75 and 16.01 mg/kg, respectively, showing higher coefficient of variation for oleanolic acid.

The highest content of oleanolic acid was observed in oils from Lechin de Granada and Dolce Agogia cultivars with a mean concentration of 78.83 and 62.25 mg/kg, respectively,

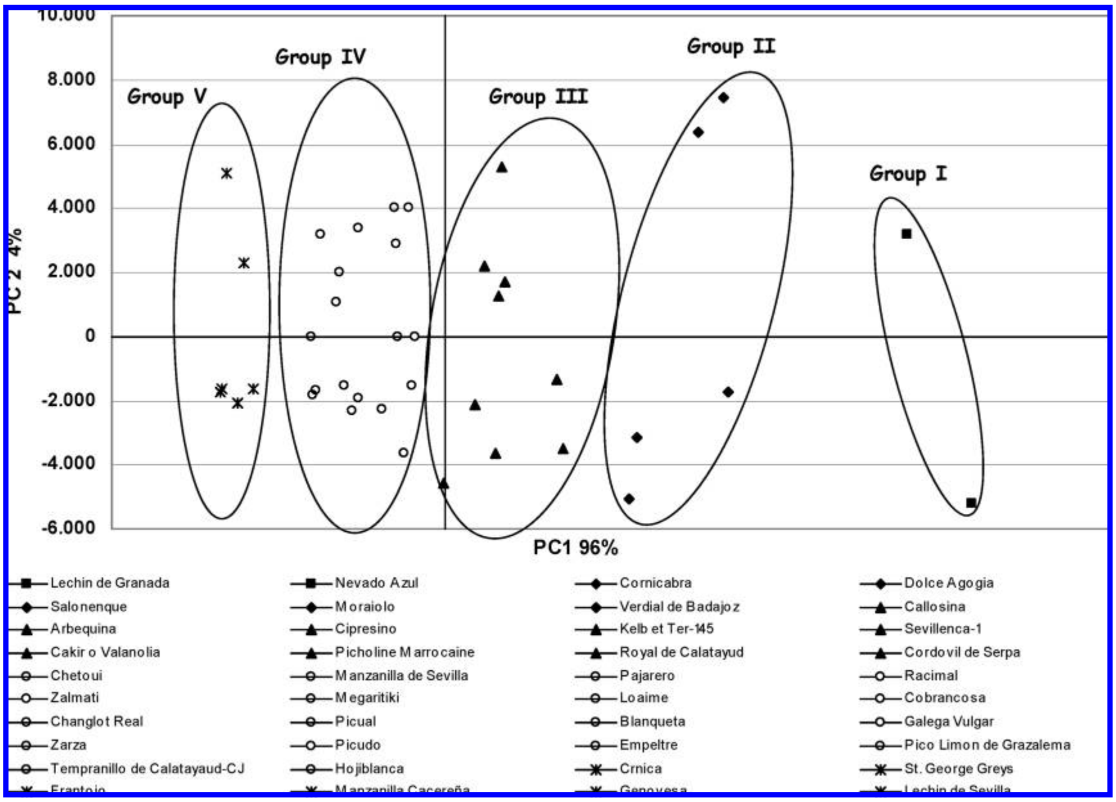


**Figure 1.** Principal component analysis (PC1 vs PC2) of virgin olive oils obtained from 40 cultivars of the World Olive Germoplasm Bank Collection of Cordoba (Spain) using the triterpenic dialcohols.

while the lowest content (3.39 mg/kg) was found in oils from Pajarero. However, higher concentrations of maslinic acid were observed in Zarza and Dolce Agogia oils, 49.81 mg/kg and 45.88 mg/kg, respectively, whereas Pico Limon de Grazalema oils showed the lowest content (3.93 mg/kg).

When these data were submitted to principal component analysis, oleanolic acid was found to contribute to 89% (PC 1) of the total variance. The residual variance (11%) was explained by maslinic acid (PC 2). Cultivar distribution and discrimination into groups according to principal component and cluster analyses are shown in **Figure 2**. The characteristics of the five groups established are described below:

Group I: Very high content of both oleanolic and maslinic acids.
Group II: High content of oleanolic acid and intermediate content of maslinic acid.
Group III: High content of both oleanolic and maslinic acids.
Group IV: Intermediate content of both oleanolic and maslinic acids.
Group V: Low content of both triterpenic acids.

The sum of the triterpenic acids (oleanolic, ursolic, and maslinic) is also shown in **Table 3**. Concentrations higher than 100 mg/kg were observed in oils from Lechin de Granada and Dolce Agogia cultivars, whereas concentrations lower than 10 mg/kg were found in Pico Limon de Grazalema and Pajarero oils. These results indicate that the genetic factor is responsible for the high variability observed for these compounds.

**Chemometrics Applied to the Triterpenic Alcohols and Acids.** On the basis of the principal component results obtained from the triterpenic alcohols and acids, a novel principal component analysis was applied to the parameters that highly contribute to the explication of the variance. The variables selected were erythrodiol, oleanolic, and maslinic acids. Two main principal components were required to capture 95% of variance between cultivars. The first principal component (PC1) explained most of the variance observed (73%) and was related mainly to oleanolic acid and erythrodiol ($r = 0.687$ and $r = 0.623$, respectively). Maslinic acid also contributed to PC1 ($r = 0.374$). The second principal component (PC 2) accounted for 22% of total variance and was related to erythrodiol ($r = 0.753$), showing an inverse correlation with both oleanolic ($r = -0.397$) and maslinic ($r = -0.525$) acids. The inclusion of additional principal components failed to improve clustering between cultivars. The distribution of oil samples according to principal component and cluster analyses is shown in **Figure 3**. The characteristics of the groups established were as follows:

Group I: Cultivars with very high content of oleanolic acid, maslinic acid, and erythrodiol (Dolce Agogia and Lechin de Granada).
Group II: Cultivars with high content of oleanolic acid, maslinic acid, and erythrodiol (Cornicabra, Zalmati, Salonenque, Sevillenca, and Zarza).
Group III: Cultivars with intermediate content of both oleanolic and maslinic acids, and very high content of erythrodiol (Nevado Azul, Moraiolo, and Verdial de Badajoz).
Group IV: Cultivars with intermediate content of oleanolic acid, maslinic acid, and erythrodiol (Chetoui, Callosina, Racimal, Megaritiki, Cobrancosa, Changlot Real, Picual, Arbequina, Cipresino, Kelb Et Ter-145, Cakir o Valanolia, Picholine Marrocaine, Royal de Calatayud, Picudo, Cordovil de Serpa, Empeltre, Tempranillo de Calatayaud, and Hojiblanca).
Group V: Cultivars with low content of oleanolic acid, maslinic acid, and erythrodiol (Lechin

**Table 3.** Triterpenic Acid Composition of the 40 Monovarietal Virgin Olive Oils Obtained from Cultivars of the World Olive Germoplasm Bank Collection of Cordoba (Spain)[a]

| cultivar | group | oleanolic acid (mg/kg) | ursolic acid (mg/kg) | maslinic acid (mg/kg) | total (mg/kg) |
|---|---|---|---|---|---|
| Lechin de Granada | I | 78.83 ± 5.42 | 1.06 ± 0.20 | 32.48 ± 2.82 | 112.36 ± 2.40 |
| Dolce Agogia | I | 62.25 ± 32.15 | 3.13 ± 1.99 | 45.88 ± 7.30 | 111.26 ± 41.44 |
| Cornicabra | II | 40.06 ± 2.95 | n.d | 22.47 ± 3.23 | 62.52 ± 6.18 |
| Sevillenca-1 | II | 38.95 ± 0.94 | 1.47 ± 0.46 | 21.18 ± 4.45 | 61.59 ± 3.05 |
| Solonenque | III | 34.37 ± 5.62 | 0.84 ± 0.54 | 40.62 ± 2.28 | 75.83 ± 2.81 |
| Zarza | III | 31.77 ± 3.33 | 2.83 ± 0.93 | 49.81 ± 4.78 | 84.40 ± 7.18 |
| Zalmati | III | 24.15 ± 0.07 | 4.07 ± 3.12 | 32.94 ± 1.70 | 61.16 ± 4.75 |
| Picholine Marrocaine | IV | 26.79 ± 21.71 | 1.77 ± 1.78 | 11.39 ± 7.61 | 39.95 ± 31.10 |
| Kelb-Et Ter 145 | IV | 25.44 ± 7.42 | 1.00 ± 0.55 | 15.09 ± 10.18 | 41.53 ± 17.05 |
| Megaritiki | IV | 21.38 ± 14.09 | 1.64 ± 0.06 | 18.18 ± 4.91 | 41.19 ± 9.12 |
| Cobrancosa | IV | 21.53 ± 1.88 | 1.08 ± 0.30 | 25.95 ± 3.11 | 48.56 ± 4.68 |
| Moraiolo | IV | 20.13 ± 6.48 | 1.90 ± 1.23 | 15.89 ± 2.28 | 37.92 ± 2.96 |
| Cordovil de Serpa | IV | 17.07 ± 1.51 | 0.65 ± 0.04 | 9.50 ± 0.11 | 27.22 ± 1.58 |
| Changlot Real | IV | 15.36 ± 0.00 | 0.52 ± 0.06 | 16.81 ± 1.36 | 32.70 ± 1.29 |
| Empeltre | IV | 15.21 ± 2.32 | 1.17 ± 1.05 | 10.85 ± 2.74 | 27.22 ± 6.11 |
| Tempranillo de Calatayaud | IV | 14.39 ± 1.10 | 2.09 ± 1.17 | 20.38 ± 0.37 | 36.85 ± 0.32 |
| Racimal | IV | 14.01 ± 1.85 | 1.66 ± 0.28 | 13.25 ± 0.51 | 28.92 ± 1.61 |
| Galega Vulgar | IV | 14.00 ± 2.09 | n.d | 15.31 ± 1.27 | 29.32 ± 3.35 |
| Picual | IV | 12.79 ± 6.68 | 1.00 ± 0.25 | 17.03 ± 9.11 | 30.81 ± 15.54 |
| Arbequina | IV | 11.87 ± 0.62 | 3.24 ± 1.45 | 18.47 ± 3.04 | 33.58 ± 5.10 |
| Cakir o Valanolia | V | 13.24 ± 0.25 | 0.36 ± 0.08 | 8.63 ± 0.12 | 22.22 ± 0.03 |
| Callosina | V | 11.63 ± 8.08 | n.d | 13.44 ± 7.68 | 25.07 ± 15.75 |
| Nevado Azul | V | 11.42 ± 2.79 | n.d | 13.52 ± 2.79 | 24.94 ± 5.58 |
| Manzanilla Cacerea | V | 11.38 ± 4.61 | 1.23 ± 1.24 | 9.93 ± 5.30 | 22.54 ± 8.66 |
| Crnica | V | 11.36 ± 0.33 | n.d | 12.83 ± 4.06 | 24.19 ± 4.38 |
| Hojiblanca | V | 11.12 ± 0.54 | 0.72 ± 0.30 | 10.63 ± 091 | 22.47 ± 0.06 |
| Chetoui | V | 10.90 ± 1.94 | n.d | 9.67 ± 0.11 | 20.57 ± 2.04 |
| Cipresino | V | 10.80 ± 2.40 | n.d | 12.09 ± 3.27 | 22.89 ± 5.66 |
| Lechin de Sevilla | V | 8.60 ± 0.11 | 0.55 ± 0.07 | 10.53 ± 1.43 | 19.69 ± 1.39 |
| Loaime | V | 8.25 ± 0.14 | 1.56 ± 1.08 | 9.80 ± 1.40 | 19.61 ± 2.34 |
| Genovesa | V | 8.23 ± 1.17 | 0.63 ± 0.06 | 8.87 ± 0.90 | 17.73 ± 2.13 |
| Frantoio | V | 8.03 ± 5.72 | n.d | 13.81 ± 9.94 | 21.83 ± 15.67 |
| Picudo | V | 8.19 ± 2.61 | 0.68 ± 0.06 | 5.49 ± 0.68 | 14.35 ± 3.22 |
| St. George Greys | V | 7.04 ± 1.64 | n.d | 10.27 ± 0.46 | 17.31 ± 2.10 |
| Verdial de Badajoz | V | 6.57 ± 0.35 | 0.63 ± 0.16 | 5.72 ± 0.16 | 12.92 ± 0.44 |
| Royal de Calatayaud | V | 5.83 ± 1.181 | 0.56 ± 0.46 | 5.15 ± 0.91 | 11.53 ± 2.54 |
| Manzanilla de Sevilla | V | 4.94 ± 0.52 | n.d | 7.40 ± 0.38 | 12.34 ± 0.90 |
| Blanqueta | V | 4.58 ± 0.80 | 1.28 ± 0.68 | 9.22 ± 0.71 | 15.08 ± 0.76 |
| Pico Limon de Grazalema | V | 4.17 ± 0.13 | 0.81 ± 0.21 | 3.93 ± 0.21 | 8.90 ± 0.28 |
| Pajarero | V | 3.39 ± 0.86 | n.d | 5.98 ± 1.65 | 9.37 ± 2.50 |
| average of 40 cultivars ± S.E. | | 17.75 ± 1.81 | 1.38 ± 0.15 | 16.01 ± 1.24 | 34.76 ± 2.91 |
| C.V. (%) | | 91.36 | 115.97 | 69.45 | 74.95 |

[a] Mean values ± standard deviation; S.E., standard error of the mean; C.V., coefficient of variation; n.d., not detected.

de Sevilla, Genovesa, Manzanilla Cacereña, Pico Limon de Grazalema, Galega Vulgar, Blanqueta, Loaime, Frantoio, St. George Greys, Pajarero, Manzanilla de Sevilla, and Crnica).

In summary, our results report for the first time, to our knowledge, the quantitative composition of triterpenic dialcohols and acids in virgin olive oils from different olive cultivars. Data obtained showed a great variability between the oil samples despite the fact that the olive cultivars belonged to the same orchard (Olive Bank Germoplasm Collection of Cordoba, Spain) and were grown under the same climate and agriculture practice. Moreover, fruit sample harvesting and processing were performed under the same conditions. Hence, we conclude that the high variability observed in virgin olive oil triterpenic composition is solely due to genetic factors. High triterpenic content (dialcohols and acids) was obtained in oils form Lechin de Granada, Dolce Agogia, Cornicabra, and Salonenque (values ranged between 197 mg/kg and 127 mg/kg), and low concentrations were found in oils from Pico Limon de Grazalema, Genovesa, Pajarero, and St. George Greys, with a mean content of 27 mg/kg. This high genetic component provides essential information for olive breeding projects. However, the application of chemometric methods showed that the triterpenic compounds were able to discriminate between oil cultivars and therefore can be considered as a valuable tool for virgin olive oil characterization. Nevertheless, further studies are required in order to carry out a more precise characterization to know the intraspecific variability for these compounds and to investigate the influence of different parameters (agronomic and/or oil processing) on the concentration of these pentacyclic triterpenes.

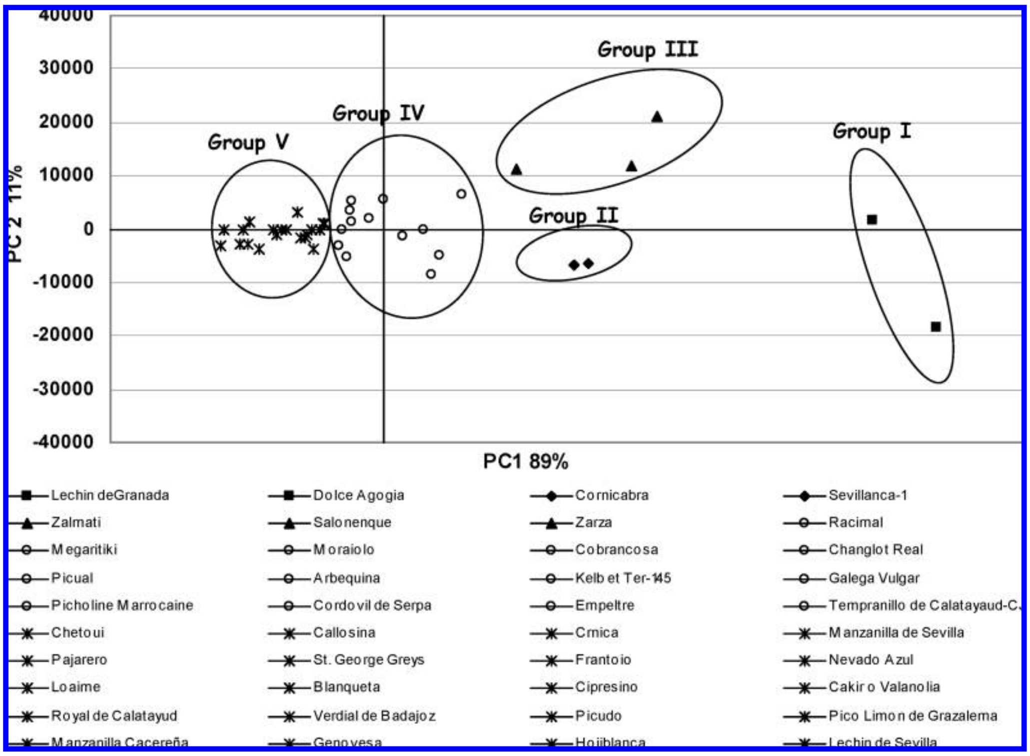


**Figure 2.** Principal component analysis (PC1 vs PC2) of virgin olive oils obtained from 40 cultivars of the World Olive Germoplasm Bank Collection of Cordoba (Spain) using triterpenic acids.

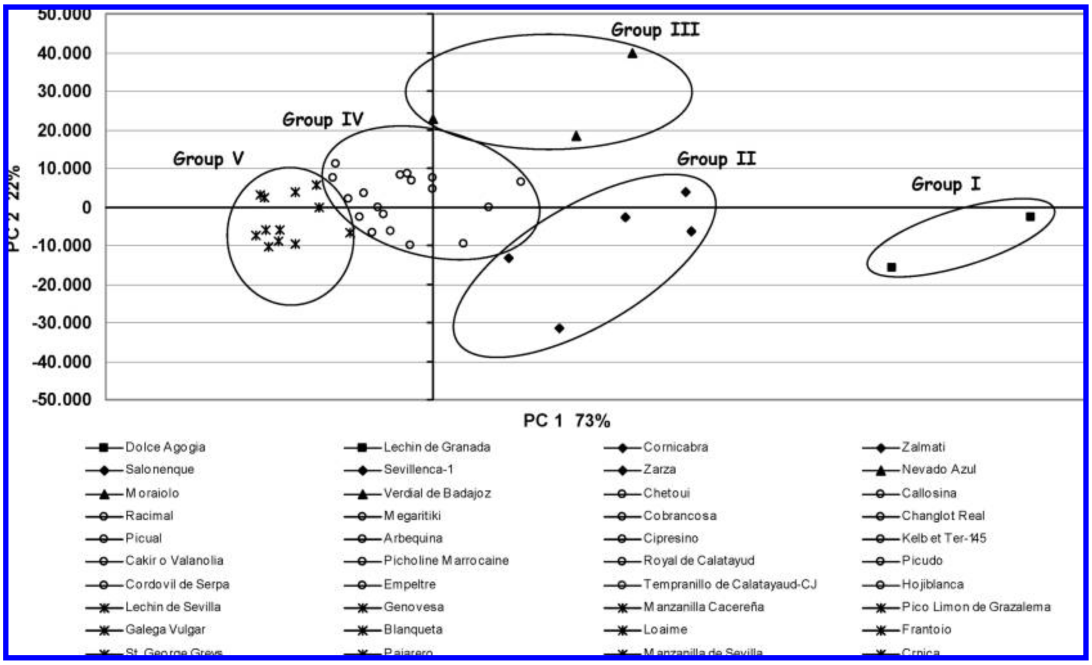


**Figure 3.** Principal component analysis (PC1 vs PC2) of virgin olive oils obtained from 40 cultivars of the World Olive Germoplasm Bank Collection of Cordoba (Spain) using values of oleanolic acid, maslinic acid, and erythrodiol.

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