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Additional Information

Unexplored olive cultivars from the Valencian Community (Spain): some chemical characteristics as a valorization strategy

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Abstract

The olive processing industry has till date been dominated by a small group of cultivars, leading to the possibility of some olive cultivars becoming extinct in the near future. In this study, we determined the composition of some chemical components in the olive oils from 31 minor olive cultivars of the Valencian Community. Our main aim was to identify suitable cultivars, which could produce differentiated olive oils, thus aiming towards their valorization. The average oil content of minor olive cultivars was found to be good, with some of them reporting approximately 60% (dry basis). On average, the total phenolic content was 229 mg kg⁻¹, with cv. Mas Blanc reporting the highest content (570 mg kg⁻¹). Among the various tocopherols found in olives, α -tocopherol was the main constituent, with a maximum concentration of 290.6 mg kg⁻¹. Linoleic acid was the main polyunsaturated fatty acid and varied between 3.4% (cv. Del Pomet) and 16.9% (cv. Blanqueta Enguera). Special attention needs to be paid to the composition of sterols, since some olive oils exceeded the limits established for some sterols by the current European legislation. Some of the cultivars studied were highly productive, and originated differentiated olive oils with a rich composition of antioxidants and essential fatty acids. In some cases, these beneficial compounds were higher than those of commercial oils obtained from the most common cultivars worldwide. These results could contribute to the commercial exploitation of some of the studied cultivars.

Keywords Minor cultivars valorization · *Olea europaea* L. · Olive oil · Characterization · Legislation · Cultivar discrimination

Introduction

The cultivation of olives has increased tremendously in the last decades as a consequence of the increased demand for olive oil and table olives among consumers. Because of the advances in agronomic practices, the yield of *Olea europaea* L. was rampant in recent times. In modern olive groves, like super intensive or hedgerow olive orchards, some olive cultivars have not been able to adapt to this type of cultivation. To ensure the intensive growth of olives, good cultivars are needed: cvs. Arbequina and Arbosana (the Spanish cultivars), cv. Leccino (the Italian cultivar), cv. Koroneiki (the Greek cultivar) are some of the prominent examples; they have been specifically developed to adapt to these cultivation conditions [1]. Several ancient and traditional olive cultivars cannot be grown in new olive orchards as they fail to proliferate in these cultivation conditions. In many other parts of the world, farmers have removed the old trees in traditional/ low density olive orchards. New foreign olive cultivars

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have been planted in these orchards because they are more productive and profitable in terms of yield. Consequently, the traditional and minor olive cultivars have almost vanished from olive orchards in several regions in the world. In Spain, about 262 cultivars were identified and classified into four main groups: major, secondary, dispersed, and local. Among the 262 cultivars, there were only 24 major cultivars [2]. At the beginning of the twenty-first century, cvs. Picual, Arbequina and Hojiblanca [3] were the only cultivars grown in more than 90% of new olive orchards in Spain. Due to the massive cultivation of these cultivars, olive germplasm was significantly reduced. The cv. Arbequina is one of the most representative olive cultivars in Spain and in other parts of the world. In fact, it is grown in almost all olive producing countries [4]. However, several traditional olive cultivars are considered local, dispersed, secondary, or minor olive cultivars. These olive cultivars have the potential to be widespread based on their productivity: the quality and chemical composition of the olive products from these cultivars is usually taken into account for growing them on a large scale. In recent years, researchers have performed chemical characterizations of these minor olive cultivars. These researchers wanted to use this information to valorize them in Tunisia [5], Greece [6], Spain [7], and around the world [8]. In the Valencian Community (Spain), recent studies were conducted to determine the quality parameters and some components of olive oil from 45 olive cultivars [9]. According to Ruiz-Domínguez et al., “a greater diversity exists in the economically less important varieties, indicating that selection among them for specific composition profiles with desirable properties... can be of interest for the recovery of neglected varieties” [9]. In the present study, we emphasized the characterization of minor cultivars. Moreover, the potentialities of this information were also explored in this study.

Apart from their quality, the minor composition of olive oils plays an important role in the overall properties of the final product. Therefore, the determination of minor components such as sterols, tocopherols and phenolic compounds, as well as of fatty acids is imperative to assess the potentialities of olive oils [10].

The main aim of the present work is to contribute to the literature of minor olive cultivars. In this study, we assessed whether minor olive cultivars could be used as important assets in olive production for the creation of differentiated olive oils. Therefore, the fat content concerning different olive cultivars was determined, and we also determined some chemical components (fatty acids, sterols, α -tocopherol, and total phenols content) of 31 minor olive cultivars from the Valencian Community. The sole purpose of the analyses was to valorize these minor cultivars and promote their cultivation.

Materials and methods

Plant material collection

In the present study, minor olive cultivars were obtained from the Valencian Community in Spain to chemically characterize their olive oils. In order to identify the olive cultivars, we used the system established by the International Union for the Protection of New Varieties of Plants (UPOV guidelines TG/99/4). After identifying all the cultivars, we compared them with standard cultivars present in the collection fields of the Universitat Politècnica de València. From all the collected material, some olive cultivars were selected. The selected cultivars were included in the collection for future research and for comparisons of the agronomic behavior. In total, 31 olive cultivars were selected: cvs. Aguilar, Blanqueta Enguera, Borriolenca, Cabaret, Callosina, Carasqueña, Cuquillo, Changlot Real, Del Patró, Del Pomet, Figuereta, Genovesa, Gileta, Grossal, Lloma, Llumero, Marfil, Mas Blanc, Millareja, Monteaguda, Morons, Morruda, Negra, Piñonera, Romana, Rotja, Rufina, Seniero, Valentins, Vallesa, and Vera. Olives from the 31 cultivars were collected throughout the Valencian Community. As shown in Fig. 1, the olives were collected in 11 municipalities. All olives were allowed to grow till they reached their optimal ripening stage, a maturation index of 2–3, according to the procedure proposed by Hermoso et al. [11]. Approximately 10 kg of olives were handpicked from each cultivar; then, they were immediately transported to the laboratory.

Olive oils extraction, moisture, and oil content determination

After harvesting the olives, the olive oil was extracted within the first 24 h. The extraction was carried out by following the methodology described by Malheiro et al. [12]: olive oils were extracted in an Abencor analyzer (Comercial Abengoa S.A., Seville, Spain) with three main units: a mill, a thermobeater (malaxation of olive pastes under controlled temperature conditions), and a centrifuge. Olive samples ($n = 5$ per cultivar) were milled with a hammer miller, and the resultant olive paste was homogenized. About 700 g of olive paste was transferred to the thermobeater unit, where malaxation was carried out for 30 min in a thermostatic water bath at 25 °C. In the final 5 min of each malaxation, 100 mL of water at 35 °C was added to improve the extraction of olive oil. In the centrifugation unit, the mixture was centrifuged for 1 min at 3500 rpm and decanted. The olive oil was collected and stored in 100-mL dark bottles.

In order to determine the fat content in the olive paste, the oil was extracted from the paste in a Soxtec Avanti



Fig. 1 Location of 31 minor olive cultivars from the Valencia Community (Spain)

2050 automatic extraction system (Foss Tecator, Höganäs, Sweden). The extraction was carried out by following the procedure described in the Annex XV of the Commission Regulation No. 2568/91 and further amendments [13]. Moisture content was determined by desiccation at 105 °C for 24 h.

Fatty acids determination

The fatty acids profile was determined by following the standard UNE55,037–73. The samples were analyzed in a GC 8000Top (CE Instruments Ltd.), which was equipped with a flame ionization detector (FID); the samples were injected into a Supelcowax 10 column (30 mm × 0.25 mm) (Sigma-Aldrich). Helium was the carrier gas at a flow of 1 mL/min. The temperatures of the injector and detector were fixed at 250 °C and 270 °C, respectively; an injection volume of 1 µL was used for sample analysis. The oven temperature was programmed at 120 °C during the first 3 min with an increase of 4 °C/min until 220 °C. The relative percentage of each fatty acid was determined by conducting an internal normalization of the chromatographic peak areas. A standard mixture of fatty acids methyl esters (Supelco37 FAME Mix) was used for identification and calibration purposes.

Sterols and tocopherols determination

In order to analyze sterols and tocopherols, we used 100 mg of fat with a known weight of an internal standard (5,7-dimethyltolcol), prepared following the technique developed by Slover et al. [14]. Saponification was performed with aqueous potassium hydroxide. The unsaponifiable fraction was extracted with cyclohexane and the solvent was removed with a stream of nitrogen. The derivatization was performed with *bis*(trimethylsilyl)-trifluoroacetamide plus 1% of trimethylchlorosilane with pyridine [14]. The derivatized total unsaponifiable fraction was injected in the same equipment used for fatty acids analysis; the analyses were carried out in a Tracer TR Sterol column (30 m × 0.22 mm; Teknokroma, Spain). The temperatures of the injector and detector were fixed at 290 °C and 300 °C, respectively. The oven temperature was maintained at 265 °C, and a sample volume of 1 µL was injected into the equipment.

Total phenolic content determination

The procedure was carried out using the analytical methodology described by Sousa et al. [15]; however, slight modifications were introduced in the procedure. Briefly, 2.5 g of olive oil were diluted (1:1 w/v) with *n*-hexane; the oil was extracted thrice with 2.5 mL of methanol/water (80:20; v/v). The mixture was centrifuged for 5 min at 5000 rpm. From

the combined extract, 1 mL was added to the same amount of Folin–Ciocalteu reagent and Na₂CO₃ (7.5%). Then, 7 mL of purified water was added to this solution mixture. After homogenization, the solution mixture was stored overnight. Finally, a spectrophotometric analysis was performed at $\lambda = 765$ nm.

For quantification purposes, a calibration curve of caffeic acid in methanol was prepared in the concentration range of 0.04–0.18 mg/mL. The final results were expressed as mg of caffeic acid equivalents per kg of olive oil (mg CAE/kg).

Statistical analysis

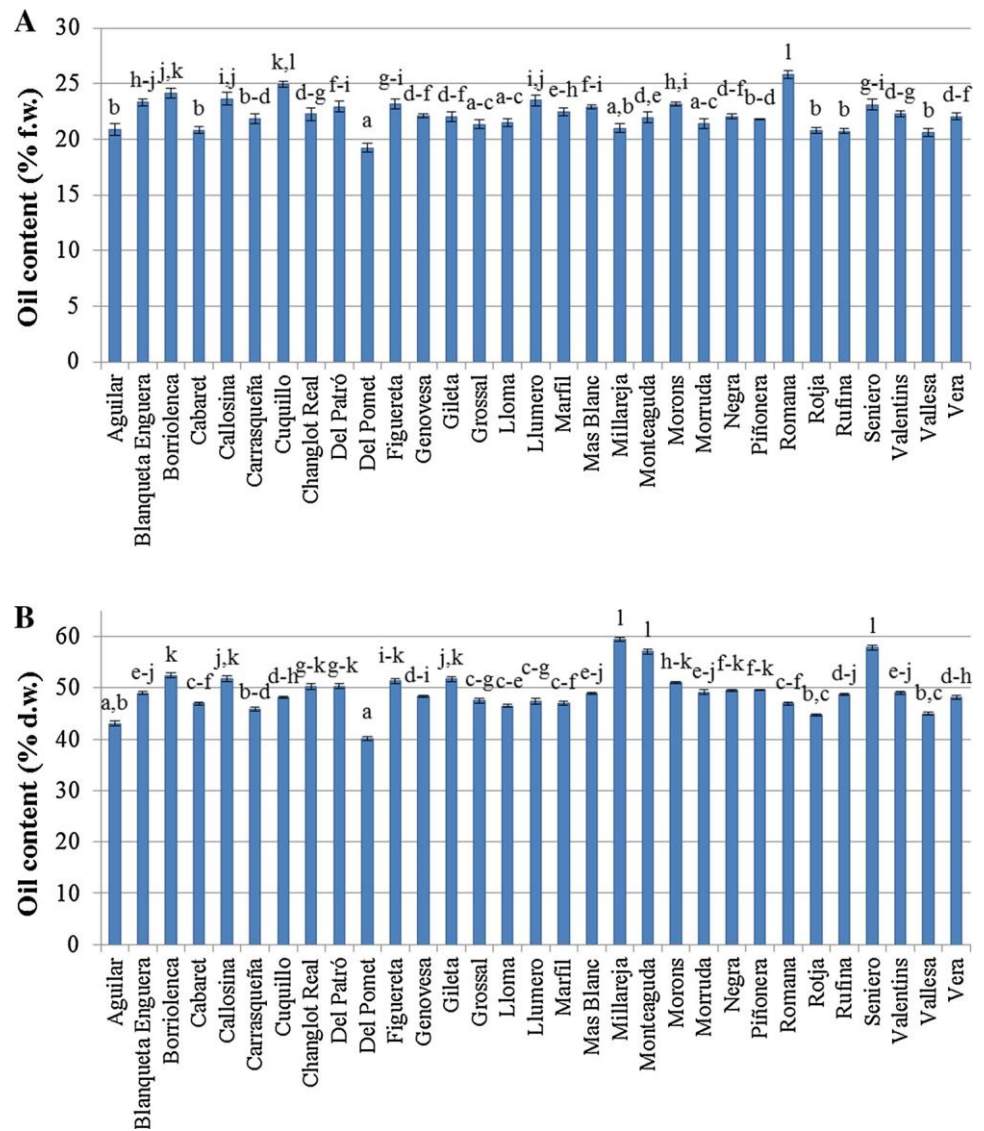
An analysis of variance (ANOVA), a principal component analysis (PCA), and a linear discriminant analysis (LDA) were carried out according to the procedure described by Limón et al. [16]. Statistical analyses were performed using SPSS software, version 22.0 (IBM Corporation, New York, USA).

Results and discussion

Oil content

Figure 2a, b presents the results of the oil content obtained in both fresh and dry weight of olives, respectively. The results differed significantly for all the 31 olive cultivars ($p < 0.001$). In fresh weight, the values varied between 19.2% (cv. Del Pometa) and 25.8% (cv. Romana) (Fig. 2a). In some cases, our results were comparable to those obtained by Ruiz-Domínguez et al. [9] as fresh weight values varied between 7.6 and 23.8%. In terms of dry weight, cv. Del Pometa showed a significantly lower content of 40.1%, while cv. Millareja reported the highest value of 59.4% of dry weight (Fig. 2b). The results indicate that some minor cultivars have high oil content, including cvs. Millareja (59.4%), Seniero (57.8%), and Monteaguda (57.0%). Compared to Spanish and worldwide important olive cultivars, higher values were reported: cv. Arbequina (53.4% d.w. at harvest moment) [17]; and cv. Coratina (20.5%) [18]. The oil content of cv. Romana was quite similar to those reported for cv. Koroneiki cultivated in Tunisia (25.4%) [19]. Therefore, the minor olive cultivars of Valencia have high oil content despite being produced in traditional olive orchards; consequently, these minor olive cultivars should be considered in new olive orchards. For these results to be validated, we should compare these minor cultivars with standard and reference olive cultivars. It is important to note that standard and reference olive cultivars must be grown in the same agroclimatic conditions. Furthermore, they should also be harvested at the same ripening stage, and must follow the same agricultural practices and processing techniques.

Fig. 2 Oil yield (**a** % in fresh weight; and **b** % in dry weight) of the 31 minor olive cultivars from Valencia Community (a–l mean values with different letters differ significantly, $p < 0.05$)



Fatty acids composition

Table 1 presents the fatty acid profile of the 31 olive cultivars. As expected, oleic acid ($C_{18:1}$) was the most abundant fatty acid. Ranging from 62.0% (cv. Blanqueta Enguera) to 84.0% (cv. Carrasqueña), the content of oleic acid varied significantly among the 31 cultivars ($p < 0.001$). Oleic acid was followed by palmitic acid ($C_{16:0}$) whose proportion varied between 7.69% (cv. Lloma) and 18.6% (cv. Blanqueta Enguera) among the 31 different cultivars. The third most abundant fatty acid was linoleic acid ($C_{18:2}$). This fatty acid, together with linolenic acid ($C_{18:3}$), is very important because they are both essential fatty acids [20]. Humans cannot synthesize these fatty acids naturally; however, they are essential to health and must be included in our diet. The proportion of linoleic acid varied from 3.43% (cv. Del Pomot) to 16.9% (cv. Blanqueta Enguera) among the 31 cultivars

included in this study. Furthermore, cv. Blanqueta Enguera was a good source of polyunsaturated fatty acids (PUFA) as it contained 17.5% of PUFA. Since PUFA content is higher in cv. Blanqueta Enguera, the stability of the oil extracted will be affected to some extent. This is because PUFA are the main substrates in the autoxidation process, which leads to the chemical deterioration of the oil. If olive oils are extracted from cv. Blanqueta Enguera, then they must be consumed immediately after extraction, as their quality deteriorates due to oxidation if stored for longer periods. Note that it is beneficial to consume this olive oil as it contains good amounts of antioxidants, namely, phenolic compounds, tocopherols, and sterols (Table 2).

Our results are in line with those obtained by Ruiz-Domínguez et al. [9] however, with great discrepancy observed in the results regarding cv. Gileta. For example, we detected lower amounts of PUFA and lower amounts of

Table 1 Characterization of fatty acids composition (%) of monovarietal olive oils, which were obtained from 31 minor olive cultivars from Valencia

tivar	C _{16:0}	C _{16:1}	C _{17:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	C _{20:0}	C _{22:0}	SFA	MUFA	PUFA
Aguilar	13.3 ± 0.10i-k	1.42 ± 0.03n,o	0.18 ± 0.01 h	2.25 ± 0.01j-m	79.2 ± 0.26n,o	3.93 ± 0.09a,b	0.64 ± 0.01f-h	0.40 ± 0.02o-q	0.15 ± 0.01j-l	16.3 ± 0.09l,m	80.6 ± 0.26n,o	4.56 ± 0.10a,b
Blanqueta Enguera	18.6 ± 0.10o	1.52 ± 0.03o	0.22 ± 0.01i,j	2.00 ± 0.02f-h	62.0 ± 1.10a	16.9 ± 0.10o	0.60 ± 0.01e-h	0.39 ± 0.01l-p	0.12 ± 0.01 g-k	21.4 ± 0.12q	63.5 ± 1.12a	17.5 ± 0.10q
Borriolenca	10.8 ± 0.12e-g	0.77 ± 0.02 h,i	0.08 ± 0.01b,c	2.09 ± 0.07h-j	77.9 ± 0.42l-n	6.46 ± 0.06e-g	0.52 ± 0.01b-e	0.39 ± 0.02m-p	0.08 ± 0.00a-c	13.5 ± 0.15f	78.7 ± 0.43k-n	7.00 ± 0.06f-i
Cabaret	11.0 ± 0.20e-g	1.95 ± 0.01p,q	0.04 ± 0.01a	1.91 ± 0.02e-g	76.8 ± 0.16k,l	9.50 ± 0.62j	0.73 ± 0.03j,k	0.29 ± 0.01c-e	0.07 ± 0.01a-c	13.4 ± 0.23e-f	78.7 ± 0.16k-n	10.2 ± 0.6l,m
Callosina	15.9 ± 0.38m,n	2.18 ± 0.12r	0.15 ± 0.02e-g	1.95 ± 0.13f-h	72.1 ± 4.14e-g	8.50 ± 0.50 h,i	0.62 ± 0.13f-h	0.34 ± 0.02f-i	0.09 ± 0.01a-e	18.4 ± 0.35o	74.2 ± 4.17e-h	9.12 ± 0.58j,k
Carrasqueña	8.76 ± 0.52c	0.52 ± 0.04b-d	0.04 ± 0.01a	2.24 ± 0.01j-m	84.0 ± 0.35q	4.02 ± 0.01a,b	0.45 ± 0.01a,b	0.33 ± 0.01f-h	0.11 ± 0.01e-i	11.5 ± 0.52b,c	84.5 ± 0.37q	4.47 ± 0.01a,b
Cuquillo	13.4 ± 0.45j,k	1.32 ± 0.01m,n	0.05 ± 0.01a,b	2.27 ± 0.01k-m	75.9 ± 0.43j-l	5.87 ± 0.23c-e	0.87 ± 0.02l	0.35 ± 0.01h-k	0.11 ± 0.01e-i	16.2 ± 0.45k-m	77.2 ± 0.43i-k	6.73 ± 0.24e-g
Changlot Real	11.4 ± 0.30f,g	0.51 ± 0.02a-c	0.16 ± 0.01f-h	2.28 ± 0.00k-m	77.1 ± 0.34l,m	6.88 ± 0.24 g	0.57 ± 0.01c-g	0.40 ± 0.01n-p	0.10 ± 0.00c-g	14.4 ± 0.32 g	77.6 ± 0.34j-l	7.45 ± 0.24 h,i
Del Patró	13.6 ± 0.23k	1.87 ± 0.02p	0.14 ± 0.02e-g	1.58 ± 0.01b,c	72.6 ± 0.02e-h	9.44 ± 0.13j	0.50 ± 0.02b,c	0.28 ± 0.01b-d	0.11 ± 0.01e-i	15.8 ± 0.23k,l	74.5 ± 0.04f-h	9.94 ± 0.13l
Del Pometa	12.6 ± 0.09 h,i	1.24 ± 0.02l,m	0.04 ± 0.01a,b	1.83 ± 0.01d-f	79.4 ± 0.43n,o	3.43 ± 0.03a	0.56 ± 0.01c-f	0.31 ± 0.01d-f	0.10 ± 0.00c-g	14.8 ± 0.09 g-i	80.7 ± 0.42n,o	3.99 ± 0.03a
Figuereta	7.92 ± 0.09a,b	0.43 ± 0.01a,b	0.05 ± 0.01a,b	2.30 ± 0.01k-m	79.4 ± 0.20n,o	8.95 ± 0.04i,j	0.67 ± 0.02 h,j	0.43 ± 0.01q,r	0.16 ± 0.01l	10.9 ± 0.10a,b	79.8 ± 0.20m-o	9.62 ± 0.03k,l
Genovesa	11.4 ± 0.36 g	0.62 ± 0.04d-f	0.19 ± 0.01 h,i	2.35 ± 0.01m,n	77.5 ± 0.09l-n	6.65 ± 0.01f,g	0.60 ± 0.00e-h	0.41 ± 0.00p,q	0.10 ± 0.00c-g	14.5 ± 0.36 g,h	78.2 ± 0.06k-m	7.25 ± 0.01 g-i
Gileta	10.6 ± 0.12e,f	1.14 ± 0.01k,l	0.17 ± 0.01 g,h	1.77 ± 0.04d,e	69.7 ± 0.43c,d	12.5 ± 0.13m	0.96 ± 0.02m	0.31 ± 0.01d-f	0.06 ± 0.01a	13.0 ± 0.10e,f	70.8 ± 0.42c,d	13.5 ± 0.13p
Grossal	12.4 ± 0.47 h	0.59 ± 0.02c-e	0.26 ± 0.01j	2.35 ± 0.01m,n	71.8 ± 0.32d-g	11.1 ± 0.01l	0.67 ± 0.01 h,j	0.45 ± 0.01r	0.15 ± 0.01k,l	15.6 ± 0.46k,l	72.3 ± 0.32c-e	11.8 ± 0.01o
Lloma	7.69 ± 0.14a	0.40 ± 0.03a	0.12 ± 0.04d,e	2.34 ± 0.03l-n	83.8 ± 0.53q	5.26 ± 0.04c	0.39 ± 0.02a	0.35 ± 0.01 g-j	0.14 ± 0.05i-l	10.7 ± 0.09a	84.2 ± 0.55q	5.64 ± 0.03c,d
Llumero	10.6 ± 0.16e	0.60 ± 0.01c-e	0.06 ± 0.00a-c	1.78 ± 0.08d,e	80.5 ± 0.32o,p	5.64 ± 0.02c,d	0.79 ± 0.01k	0.35 ± 0.00h-k	0.12 ± 0.01f-i	12.9 ± 0.15e,f	81.1 ± 0.31o,p	6.42 ± 0.02e,f
Marfil	11.0 ± 0.56e-g	0.60 ± 0.01c-e	0.14 ± 0.02e-g	1.73 ± 0.03c,d	78.9 ± 0.57m-o	6.13 ± 0.13d-f	0.77 ± 0.02k	0.25 ± 0.01a	0.08 ± 0.01a-c	13.2 ± 0.56e-f	79.5 ± 0.57l-o	6.90 ± 0.12f-h
Mas Blanc	10.8 ± 0.11e-g	1.20 ± 0.01k,l	0.03 ± 0.01a	1.34 ± 0.01a	74.8 ± 0.30i-k	11.4 ± 0.05l	0.47 ± 0.02b	0.27 ± 0.01a-c	0.14 ± 0.01h-l	12.6 ± 0.09d,e	76.0 ± 0.31h-j	11.9 ± 0.05o
Millareja	13.5 ± 0.17k	0.97 ± 0.05j	0.13 ± 0.02d-f	2.37 ± 0.01m,n	70.6 ± 0.42c,e	11.5 ± 0.32l	0.75 ± 0.08k	0.36 ± 0.02i-l	0.09 ± 0.01a-e	16.5 ± 0.16m	71.5 ± 0.40c,d	12.2 ± 0.39o
Monteaguda	16.4 ± 0.32n	1.23 ± 0.02l,m	0.12 ± 0.01d,e	2.67 ± 0.07o	71.5 ± 0.35d,f	7.07 ± 0.05 g	0.59 ± 0.03d-h	0.38 ± 0.01k-o	0.09 ± 0.01a-e	19.7 ± 0.26p	72.8 ± 0.37d-f	7.66 ± 0.07i
Morons	9.59 ± 0.02d	0.65 ± 0.02e-g	0.14 ± 0.01e-g	1.85 ± 0.02d-f	73.8 ± 0.20 g-i	13.6 ± 0.10n	0.64 ± 0.01 g-i	0.25 ± 0.01a	0.08 ± 0.01a-c	12.0 ± 0.04c,d	74.4 ± 0.21e-h	14.2 ± 0.09p
Morruda	12.4 ± 0.31 h	0.85 ± 0.10i	0.06 ± 0.01a-c	2.68 ± 0.29o	72.8 ± 0.49f-i	10.4 ± 0.85k	0.53 ± 0.03b-e	0.41 ± 0.01p,q	0.12 ± 0.01f-i	15.8 ± 0.46k,l	73.7 ± 0.43e-g	10.9 ± 0.87m,n
Negra	10.8 ± 0.15e-g	0.47 ± 0.02a,b	0.07 ± 0.01a-c	3.82 ± 0.01q	73.3 ± 0.26f-i	11.0 ± 0.34k,l	0.58 ± 0.01c-g	0.43 ± 0.02q,r	0.12 ± 0.01f-i	15.2 ± 0.11h-j	73.8 ± 0.27e-g	11.6 ± 0.34n,o
Piñonera	15.4 ± 0.05l,m	1.49 ± 0.02o	0.09 ± 0.01c,d	1.74 ± 0.01c,d	69.2 ± 0.26c	11.0 ± 0.02k,l	0.72 ± 0.01i-k	0.32 ± 0.01e-g	0.08 ± 0.01a-d	17.7 ± 0.05n,o	70.6 ± 0.26c	11.7 ± 0.02o
Romana	18.0 ± 0.06o	2.05 ± 0.02q	0.04 ± 0.01a	2.19 ± 0.02i-l	68.5 ± 0.23b,c	8.23 ± 0.05 h	0.59 ± 0.01d-h	0.35 ± 0.01h-k	0.09 ± 0.01b-f	20.7 ± 0.07q	70.6 ± 0.23c	8.83 ± 0.06j
Rotja	14.9 ± 0.51l	1.09 ± 0.16k	0.12 ± 0.03d,e	2.04 ± 0.09 g-i	66.8 ± 0.82b	9.23 ± 0.66j	0.62 ± 0.02f-h	0.32 ± 0.01e-g	0.07 ± 0.01a,b	17.5 ± 0.50n	67.9 ± 0.81b	9.85 ± 0.64l
Rufina	12.7 ± 0.26h-j	1.12 ± 0.02k,l	0.22 ± 0.01i,j	2.71 ± 0.01o	76.8 ± 0.66k,l	5.47 ± 0.31c,d	0.61 ± 0.01f-h	0.43 ± 0.01q,r	0.12 ± 0.01 g-j	16.2 ± 0.29k-m	78.0 ± 0.64j-m	6.08 ± 0.31d,e
Seniero	8.82 ± 0.54c	0.76 ± 0.01 g-i	0.18 ± 0.01 h	1.48 ± 0.01a,b	82.8 ± 0.46q	5.93 ± 0.03c-e	0.46 ± 0.01a,b	0.26 ± 0.01a,b	0.09 ± 0.01a-e	10.9 ± 0.54a,b	83.5 ± 0.45q	6.40 ± 0.03e,f
Valentins	8.43 ± 0.34b,c	0.72 ± 0.05f-h	0.12 ± 0.04d,e	2.15 ± 0.06i-k	82.3 ± 0.70p-q	4.44 ± 0.37b	0.57 ± 0.01c-g	0.43 ± 0.01q,r	0.11 ± 0.01d-h	11.3 ± 0.33a-c	83.0 ± 0.75p,q	5.01 ± 0.37b,c
Vallesa	10.8 ± 0.17e-g	0.45 ± 0.01a,b	0.04 ± 0.01a,b	2.95 ± 0.01p	73.6 ± 0.24f-i	11.2 ± 0.26l	0.52 ± 0.01b-d	0.36 ± 0.01i-m	0.14 ± 0.01h-l	14.4 ± 0.17 g	74.0 ± 0.24e-h	11.8 ± 0.27o
Vera	12.5 ± 0.31 h	0.97 ± 0.02j	0.05 ± 0.01a,b	2.48 ± 0.01n	74.2 ± 0.39h-j	6.10 ± 0.10d-f	0.47 ± 0.02b	0.37 ± 0.01j-n	0.08 ± 0.01a-c	15.5 ± 0.30i-k	75.2 ± 0.40 g-i	6.57 ± 0.12e-g
P value	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^B	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A

^{a-1}In the same column mean values with different letters differ significantly ($p < 0.05$)

^A $p < 0.05$, by means of Levene's test. p values are obtained from one-way Welch ANOVA analysis. Means were compared by Dunnett T3's test, since equal variances could not be assumed

^B $p > 0.05$, by means of Levene's test. p values are obtained from one-way ANOVA analysis. Means were compared by Tukey's test, since equal variances could be assumed

Table 2 Sterols (g 100 g⁻¹ of sterols%), α -tocopherol (mg kg⁻¹ of oil), and total phenols content (mg kg⁻¹ of oil) of monovarietal olive oils obtained from 31 minor olive cultivars from Valencia

Cultivar	Apparent β -sitosterol	Campesterol	Stigmasterol	α -Tocopherol	Total phenols content
Aguilar	95.0 \pm 0.14d–j	3.1 \pm 0.02d–f	1.6 \pm 0.40i	139.2 \pm 0.91d	170 \pm 1.17k
Blanqueta Enguera	94.2 \pm 0.12b–e	4.3 \pm 0.05k	0.6 \pm 0.08a–c	171.6 \pm 1.50h–j	321 \pm 1.19t
Borriolenca	91.5 \pm 0.44a	4.3 \pm 0.06k	1.4 \pm 1.23f–i	61.0 \pm 0.15a	121 \pm 1.44b
Cabaret	93.7 \pm 0.10b,c	3.8 \pm 0.04i,j	0.4 \pm 0.01a	198.3 \pm 0.83m	296 \pm 0.349q
Callosina	95.8 \pm 0.21i–l	2.7 \pm 0.26a–c	0.4 \pm 0.01a	255.3 \pm 2.93o	151 \pm 1.09 h
Carrasqueña	94.5 \pm 0.73b–g	3.7 \pm 0.33h–j	0.7 \pm 0.01a–e	154.2 \pm 0.62e–g	192 \pm 1.50m
Cuquillo	96.2 \pm 0.35k,l	3.1 \pm 0.04c–f	0.7 \pm 0.05a–e	342.9 \pm 0.93r	200 \pm 1.10n
Changlot Real	95.4 \pm 0.61f–l	2.8 \pm 0.05b–d	1.2 \pm 0.04d–i	186.2 \pm 2.11k–m	201 \pm 0.857n
Del Patró	94.6 \pm 0.19c–h	3.9 \pm 0.02j	1.5 \pm 0.06 g–i	113.9 \pm 0.24c	122 \pm 0.210b,c
Del Pomet	94.7 \pm 0.42c–i	2.6 \pm 0.03a,b	1.4 \pm 0.03f–i	98.3 \pm 0.24b	126 \pm 0.330d
Figuereta	95.5 \pm 0.12 g–l	3.2 \pm 0.02e–g	1.3 \pm 0.24e–i	161.8 \pm 0.58 g,h	184 \pm 0.167l
Genovesa	93.6 \pm 0.11b,c	3.5 \pm 0.27 g–i	1.7 \pm 0.14i	70.7 \pm 0.33a	141 \pm 0.812f
Gileta	94.6 \pm 0.35c–h	3.3 \pm 0.04e–g	1.5 \pm 0.02 h,i	114.9 \pm 0.27c	344 \pm 0.171v
Grossal	95.0 \pm 0.63d–j	3.4 \pm 0.16f–h	1.4 \pm 0.12f–i	130.9 \pm 0.96d	301 \pm 0.558 s
Lloma	93.7 \pm 0.76b,c	3.8 \pm 0.05i,j	1.1 \pm 0.04b–i	183.9 \pm 0.49j–l	123 \pm 0.436c
Llumeró	94.3 \pm 0.64b–f	3.8 \pm 0.29i,j	0.6 \pm 0.03a–c	141.9 \pm 2.85d–f	131 \pm 0.885e
Marfil	96.2 \pm 0.35k,l	3.0 \pm 0.01c–e	0.5 \pm 0.01a	271.0 \pm 0.67p	447 \pm 0.192y
Mas Blanc	93.7 \pm 0.59b–c	2.7 \pm 0.10a,b	0.3 \pm 0.03a	187.1 \pm 0.18h–k	570 \pm 1.18z
Millareja	93.4 \pm 0.70b	3.5 \pm 0.06 g–j	0.6 \pm 0.06a–d	180.4 \pm 2.08i–l	127 \pm 0.420d
Monteaguda	94.4 \pm 0.53b–g	2.7 \pm 0.05a–c	0.5 \pm 0.03a,b	237.7 \pm 1.72n	439 \pm 1.72x
Morons	94.0 \pm 0.31b–d	3.9 \pm 0.06j	0.6 \pm 0.04a–c	168.1 \pm 0.29 g–i	168 \pm 0.115j
Morruda	95.7 \pm 0.50h–l	2.8 \pm 0.38b–d	0.8 \pm 0.14a–e	155.4 \pm 2.01f,g	325 \pm 0.751u
Negra	96.3 \pm 0.37l	2.9 \pm 0.02b–e	0.6 \pm 0.04a–d	250.4 \pm 0.99n,o	419 \pm 0.161w
Piñonera	95.1 \pm 0.06e–k	3.9 \pm 0.02j	0.8 \pm 0.01a–g	65.0 \pm 0.11a	190 \pm 0.159m
Romana	95.6 \pm 0.06 g–l	3.8 \pm 0.02i,j	0.6 \pm 0.06a–c	198.6 \pm 1.85m	99 \pm 0.149a
Rotja	95.6 \pm 0.25h–l	3.3 \pm 0.07e–g	0.9 \pm 0.06a–h	281.9 \pm 4.50p,q	143 \pm 0.152 g
Rufina	94.7 \pm 0.41c–i	3.2 \pm 0.03e–g	1.1 \pm 0.03c–i	192.5 \pm 3.24l,m	299 \pm 0.174r
Seniero	96.0 \pm 0.84i–l	2.8 \pm 0.09b–d	0.8 \pm 0.05a–g	155.7 \pm 6.08f,g	204 \pm 0.795o
Valentins	96.3 \pm 0.25k,l	2.4 \pm 0.15a	0.8 \pm 0.11a–f	140.2 \pm 0.88d,e	154 \pm 0.150i
Vallesa	91.1 \pm 1.00a	4.6 \pm 0.20k	1.6 \pm 0.43i	61.0 \pm 0.35a	130 \pm 0.323e
Vera	94.7 \pm 0.12c–h	3.0 \pm 0.06b–e	0.4 \pm 0.11a	290.6 \pm 1.23q	272 \pm 0.232p
<i>p</i> value	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A	< 0.001 ^A

^{a–t}In the same column mean values with different letters differ significantly ($p < 0.05$)

^A $p < 0.05$, by means of Levene's test. *p* values are obtained from one-way Welch ANOVA analysis. Means were compared by Dunnett T3's test, since equal variances could not be assumed

linoleic acid, 12.5% in our study against the 22.85% found by Ruiz-Domínguez et al. [9]. Considering the European legislation [13], the results obtained for myristic (C_{14:0}) (data not shown), linolenic, arachidic (C_{20:0}), eicosanoic (C_{20:1}) (data not shown), behenic (C_{22:0}), and lignoceric acids (C_{24:0}) (data not shown) were below the maximum legal limits in all the cultivars (≤ 0.03 ; ≤ 1.00 ; ≤ 0.60 ; ≤ 0.50 ; ≤ 0.20 ; and ≤ 0.20 , respectively). Nevertheless, as far as C_{14:0} is concerned, other cultivars also report higher values than the legal limits [21].

Sterols and α -tocopherol content

Sterols and tocopherols are beneficial human health [22, 23] by reducing plasma cholesterol [24], and due to their antioxidant properties. Besides, tocopherols also perform important vitaminic functions due to vitamin E [25]. Table 2 shows the amounts of sterols and α -tocopherol in the 31 olive oils. The relative percentage of the apparent β -sitosterol varied between 91.1% (cv. Vallesa) and 96.3% (cvs. Negra and Valentins), while campesterol values varied from 2.4 to 4.3%.

Furthermore, the relative percentage of stigmasterol varied from 0.3 to 1.6%. According to the values established in European legislation [13], the maximum limits of some sterols were exceeded in the olive oils obtained from cvs. Blanqueta Enguera, Borriolenca, and Vallesa. For example, cvs. Borriolenca and Vallesa have less than 93% of apparent β -sitosterol. The levels of campesterol were higher than 4% in the two aforementioned cultivars and in cv. Blanqueta Enguera, thereby exceeding the maximum limits laid down by the European legislation [13]. Nevertheless, we need to highlight that the levels reported for sterols were not obtained by the official method from Regulation (EEC) No. 2568/91 and further amendments [13]. In this sense, the results obtained are indicative and the comparison with the European legislation is not straightforward.

The levels of α -tocopherol, varied from 61 mg kg⁻¹ (cvs. Borriolenca and Vallesa) to 343 mg kg⁻¹ (cv. Cuquillo). For similar maturation indexes, cv. Cuquillo reported higher tocopherols content than seven important olive cultivars from Southern Spain (cvs. Arbequina, Carrasqueña, Corniche, Manzanilla Cacereña, Morisca, Picual, and Verdial de Badajoz). In fact, some of these olive cultivars are widely distributed in the world [26]; however, these cultivars were not grown under the same agroclimatic conditions and they were not even treated with the same agricultural and processing techniques. It is important to note that α -tocopherol accounts for 11% of the total oxidative stability of the oil [27].

Total phenols content

Phenolic compounds are an important component of olive oil because of the following factors: antioxidant properties and health benefits [28]; improvement of the oxidative stability [27, 29]; and enhancement of the olive oil sensory attributes [30]. In the olive oils extracted from the 31 minor cultivars of the Valencian Community, the average total phenols content was 229 mg kg⁻¹ (Table 2). However, the phenolic contents ranged from 99 mg kg⁻¹ (cv. Romana) to 570 mg kg⁻¹ (cv. Mas Blanc) (Table 2). Ruiz-Domínguez et al. [9] reported similar results for the total amount of phenols: the amount of total phenols varied between 55 and 646 mg kg⁻¹, with an average value of 243 mg kg⁻¹. In four of the olive cultivars under study [cvs. Negra (419 mg kg⁻¹), Monteaguda (439 mg kg⁻¹), Marfil (447 mg kg⁻¹), and Mas Blanc (570 mg kg⁻¹)], an appreciable amount of phenols was detected (> 400 mg kg⁻¹). The amount of phenolic compounds mainly depends on the following factors: olive cultivar [31], cultivation system, agricultural practices, region of cultivation [32], and maturation stage [33, 34]. In the 31 olive cultivars, the amount of phenolic compounds varied because of the aforementioned factors or due to a conjunction of some of those factors. Besides the variation

in the phenolic content of some of the studied cultivars, it should be emphasized that some of these cultivars may become extinct in the near future. Nevertheless, the phenolic content of some of these cultivars is greater than that observed in cv. Arbequina, which is one of the most common olive cultivars in Spain and in other parts of the world [35, 36]. For comparative purposes, note that these cultivars were grown under different agroclimatic conditions and they were treated with different agricultural and processing techniques; therefore, the comparison is just indicative. These olive oils are a good source of natural phenolic compounds, which enhances the olive oil sensory and bioactive properties, and consequently improves the olive oil shelf-life.

Differentiation and discrimination of olive cultivars

After determining the chemical compositions (fatty acids, α -tocopherol, total phenols content, and sterols) and the oil content of 31 monovarietal olive oils, we tried to distinguish and discriminate these oils by performing PCA and LDA. As shown in Figs. 3 and 4, these 31 olive cultivars could be differentiated and distinguished on the basis of their chemical composition and oil content, respectively. For example, cvs. Marfil, Mas Blanc, and Monteaguda were characterized by their higher phenol content, α -tocopherol, and apparent β -sitosterol. These three groups of components influence the olive oil properties. This is because all these compounds have significant antioxidant properties. Therefore, olive oils extracted from these cultivars might have higher

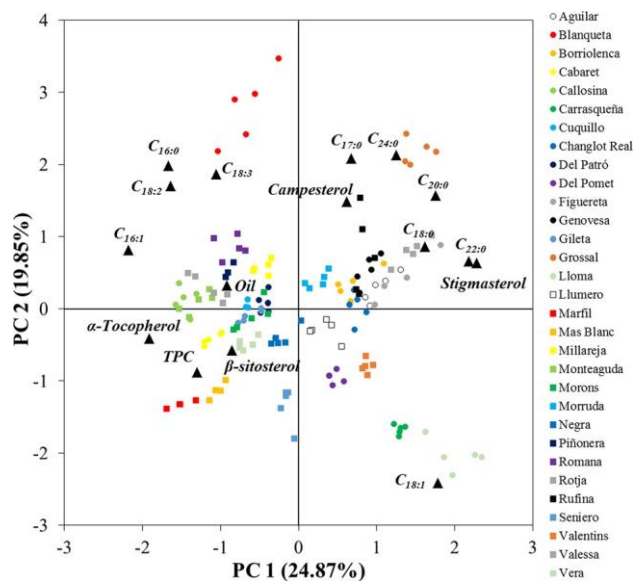


Fig. 3 Principal component analysis obtained by using the fatty acids profile, sterols, α -tocopherol, total phenols content and oil yield of the 31 minor olive cultivars from Valencia. The two principal components accounted for 44.72% of the total variance of the data

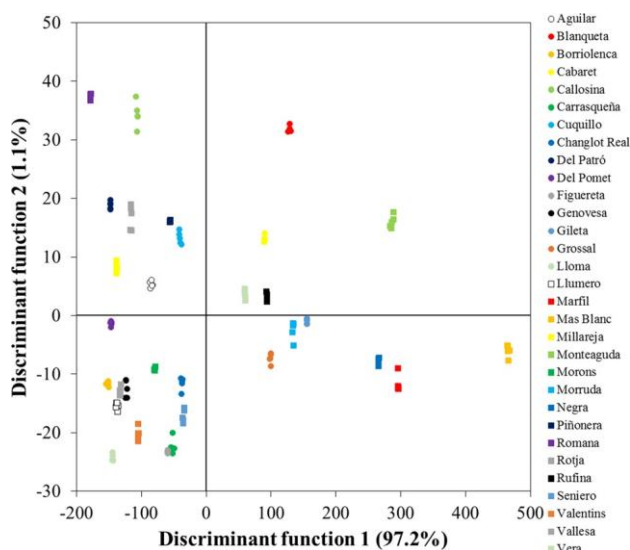


Fig. 4 Linear discriminant analysis obtained from the chemical composition of the 31 olive oils from Valencia Community. The discriminant functions accounted for 98.3% of the total variance

stability than those extracted from other cultivars, with lower amounts of these components [27]. Furthermore, these olive oils have many health benefits due to their antioxidant content. Therefore, consumers are advised to include these olive oils in their diet and avail the health benefits of antioxidants [37]. A discriminate model was developed by performing stepwise LDA, where the first two discriminant functions were associated with 98.3% of the variance of experimental data (Fig. 4). A very satisfactory performance was observed in the built model: it correctly assigned all the 31 olive oils with the original groups. In addition, it was also suitable for the cross-validation procedure (sensitivities and sensibilities of 100%). Other authors were also able to identify olive oil samples by performing LDA. In order to confirm the identity of olive oil samples, they also performed a characterization of the chemical components [9, 38–40]. This means that chemical composition can be a useful tool for the identification and differentiation of olive oil cultivars. Furthermore, the differences found among cultivars could improve the olive germplasm by increasing their diversity. This aspect may be crucial in the future, mainly in a climate change scenario, where many olive cultivars may not adapt and may reduce their production considerably, or simply perish. Concerning pests and diseases, the cultivation and introduction of minor cultivars is a good strategy to control their incidence, since some of these cultivars may be less susceptible, and some of them possibly resistant to pathogens and insect pests. Therefore, the inclusion of minor cultivars for the production of olive products may be a pool of genetic resources with advantages not only for the consumer but also regarding a series of ecological-environmental features.

Conclusions

Based on the results of the present work, the following conclusions were reached: some minor olive cultivars are suitable for producing differentiated olive oils; therefore, some of these cultivars should be cultivated on a commercial scale. The oil content (> 50% dry weight) was high for some of these cultivars, such as cvs. Milareja, Monteaguda, and Seniero. In other cultivars, significant amounts of antioxidant molecules were detected. These molecules certainly increased the value of the olive oil. The antioxidant content was the highest in the following cultivars: Marfil, Mas Blanc, and Monteaguda. In terms of sterol composition, the limits of some cultivars were greater than those established by the European legislation. This was especially pronounced for the following cultivars: Blanqueta Enguera, Borriolenca, and Vallesa. Currently, the same typical olive cultivars are planted in most orchards all over the world. By valorizing these cultivars, we can prevent their complete disappearance.

Compliance with ethical standards

Conflict of interest All authors declare that we have no conflict of interest, and the article is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Compliance with Ethics requirements This article does not contain any studies with human or animal subjects.

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