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Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad Real, Spain)

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Abstract

The potential and real qualities of virgin olive oils from the DO "Aceite Campo de Montiel" were evaluated. The regulated physicochemical and sensory parameters, the stability parameters and the fatty acid, sterol and triterpenic dialcohol compositions were analysed. The results of the regulated parameters in the potential quality study classified all the analysed oils into the "extra virgin" category. The varieties *Picual* and *Cornicabra* showed remarkably high stability, due to their high tocopherol and total polyphenol contents. Oleic and linoleic acids were the most useful fatty acids used to discriminate the predominant varieties, *Picual* and *Cornicabra*, from the others. The variety *Cornicabra* stood out due to its high campesterol content, Arbequina due to its low β-sitosterol content and high 24-methylenecholesterol, Δ5-avenasterol, Δ7-stigmastenol and total sterol contents, and *Picual* for its high sitostanol content. All the analysed samples in the real quality study were classified in the "extra virgin" category according to the regulated physicochemical parameters, but only 69% of them qualified if the sensory parameters were also taken into account. The predominance of the varieties *Picual* and *Cornicabra* in the study area was mainly responsible for the high oil stability and reflects the fatty acid and sterol compositions of the oils from the oil mills in the potential quality study.

Keywords: Virgin olive oil; Quality; Sensory; Physical–chemical; Designation of origin

1. Introduction

The designation of origin (DO) system is a way of ensuring the unique quality of products such as virgin olive oils. Granted by the European Union, this label both guarantees that the product complies with quality requirements and that its unique character is linked to the geographical and climatic characteristics of the area, where the product is traditionally elaborated. Therefore, a DO cannot be created, but rather its existence is recognised by authorities who establish the link between a name, a geographical area and a quality product (Mercacei, 2000).

Economically speaking, the products protected by a DO represent a small percentage of the final agro-food consumption in Spain (about 10%). This fact is, however, determined by the very definition and nature of these products, which cannot belong to the larger market, since their productions are necessarily limited to the characteristic geographic zone, and the yields are also restricted (Revilla, 1995).

The area of Campo de Montiel, situated in the south-eastern section of the Province of Ciudad Real (Castilla-La Mancha, Spain), shows particular orographic and pedo-climatic conditions which give special differentiating properties to their oils: high stability, intense fruity attributes (apple, tomato and other fruits) as well as balanced bitter and pungent attributes, all of which enable these oils to become eligible for protection under a DO. Furthermore, these products are of great economic and social

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importance in the area, since this is one of the few crops capable of maintaining the population in the rural environment. The Campo de Montiel area devotes about 40,000 ha to the olive crop, producing approximately 15,000 t of oil. Traditionally, the stake has been for variety duplicity with *Cornicabra* and *Picual*, even within a single plot, with the aim of improving the olive fruit setting and taking advantage of the stepped maturation, in order to obtain more balanced oils. The varieties *Manzanilla de Centro* and *Local* can also be found as single trees in the area studied, and *Arbequina* in new plantations.

On 13 February 2004, in Castellar de Santiago (Ciudad Real), the Association for the Promotion of Olive Oil from Campo de Montiel was created, with the purpose of promoting the DO "Aceite Campo de Montiel". Since then, work has been carried out for the preparation of the List of Specifications necessary for the provisional recognition of the DO. The properties of the olive oils from the zone protected by the DO are listed in that document, and we have also included them in this study.

The physicochemical and sensory properties of the oil from some Mediterranean areas have been determined in other works (Aparicio, Ferreiro, Cert, & Lazón, 1990; Beltrán, Jiménez, Aguilera, & Uceda, 2000; Conte, Carboni, & Lercker, 1993; Giacometti & Milin, 2001; Graell et al., 1993; Gracia, 2001; Motilva, Jaria, Bellart, & Romero, 1998; Motilva, Ramo, & Romero, 2001; Poiana et al., 1997; Poiana et al., 1996; Russo & Fichera, 1993; Salvador, Aranda, & Fregapane, 1998; Salvador, Aranda, Gómez-Alonso, & Fregapane, 2001; Salvo, Alfa, Lo Curto, & Dugo, 1998).

The potential quality of olive oils is reached when the raw material has been selected (olives at optimum ripening stage, free of pests, harvested directly from the tree) and has also been processed under optimum conditions (without delay, at optimal processing temperatures, with quick separation of residues and by-products), while the real quality is that found in olive oils sampled randomly from storage tanks located in different olive oil mills in the study area.

The regulated physicochemical and sensory parameters are those defined by the established regulations (Pardo, Calcerrada, & Alvarruiz, 1998). These parameters determine the existence of any defect in the oil or the existence of causes for the subsequent appearance of defects (Pardo, Tardáguila, & Gómez, 1996).

The stability parameters are those determining the commercial quality of the oils at the end of the maximum possible time of storage or shelf life (when it is bottled and distributed in supermarkets or retailers), maintaining optimum sensory properties.

The fatty acid composition has a relatively wide range due to the genetic and environmental factors which prevail during the development of the fruit, with the ripening stage of the olives at harvest time also being a factor (Kiritsakis & Markakis, 1987). The fatty acid composition has previously been used by a number of authors as a parameter for oil classification (Alonso & Aparicio, 1993; Lanza, Russo, & Tomaselli, 1998; Motilva et al., 2001).

The sterols are monovalent higher alcohols which are included in the unsaponifiable fraction of the olive oil. The composition of the steroidal fraction of the olive oil is a very useful parameter for detecting adulterations or to check authenticity, since it can be considered as its real fingerprint (Cert, Moreda, & García-Moreno, 1997; Jiménez & del Valle, 1996; Salvador et al., 1998). β-Sitosterol is the most abundant sterol in olive oil and has a recognised effect in lowering cholesterol levels by opposing its absorption in the intestinal tract (Viola, 1997).

The triterpenic dialcohols, which are also part of the unsaponifiable fraction of the olive oil, are usually analysed together with the sterol fraction. Their content is restricted by Regulation EEC 2568/91 to a maximum of 4.5% of the total sterols (EUC, 1991).

In this paper, the potential and real quality of virgin olive oil from the Campo de Montiel area was evaluated in order to obtain the DO. This was done by analysing the regulated physicochemical, sensory and stability parameters and the fatty acid, sterol and triterpenic dialcohol compositions.

2. Materials and methods

2.1. Potential quality

2.1.1. Selection of plots and trees within the area studied

Forty-one plots were selected according to the predominant variety or to the presence of a sufficient number of isolated trees of non-predominant varieties, distributed as follows: *Picual* = 15; *Cornicabra* = 15; *Manzanilla de Centro* = 5; *Arbequina* = 3; *Local* = 3. For each variety, the sampling stations were distributed throughout the study area, although with a higher number of selected plots, where the variety was found to have a higher incidence.

Within each plot, 6 typical trees from the represented variety were selected and marked. The genetic and morphological characterization and identification of the trees was done at the Cátedra de Pomología, Escuela Técnica Superior de Ingenieros Agrónomos y de Montes, Córdoba (Andalusia, Spain).

2.1.2. Sampling

Samples were taken at two precise moments: (i) the beginning of the harvest season (first fortnight of December, 2003), when most of the fruits had purple-black skin and green flesh, although some of them still had green skin, and (ii) the middle-end of the harvest season (second fortnight of January, 2004), when all the fruits were ripe, some of them even over-ripe. In both cases the sampling began with the earlier varieties. The total number of samples was 82 (41 plots × 2 collections). The results of the first and second sampling were combined for the statistical analysis.

In each plot, 10 kg of healthy olives were picked by hand from the selected trees at the beginning and at the end of the harvest season and put into net bags. The samples were labelled and taken rapidly to a pilot plant for the extraction of olive oil at the Escuela Técnica Superior de Ingenieros Agrónomos (ETSIA), Province of Albacete (Spain).

2.1.3. Olive oil extraction

An experimental oil mill comprised of a hammer mill, malaxator and centrifuge (Abencor system, Comercial Abengoa, SA, Sevilla, Spain) was used. About 2 l of oil were obtained from each sample separately, as described by Martínez, Muñoz, Alba, and Lazón (1975).

2.1.4. Analytical determinations

Determination of the regulated physicochemical quality parameters (free acidity, peroxide value and UV absorption characteristics, K_{270} and K_{232}), was carried out, following the analytical methods described by Regulation EEC/2568/91 of the Commission of the European Union (EUC, 1991).

Free acidity, given as % of oleic acid (or °), was determined by titration of a solution of oil dissolved in ethanol/ether (1:1) with 0.1 M potassium hydroxide ethanolic solution.

Peroxide value, expressed in milliequivalents of active oxygen per kilogramme of oil (meq/kg), was determined as follows: a mixture of oil and chloroform–acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution.

K_{270} and K_{232} extinction coefficients were calculated from absorption at 270 and 232 nm, respectively, with a UV spectrophotometer (Hewlett–Packard, HP 8452 A), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Sensory analysis (median of defects, median of fruity and panel classification test) of the samples was carried out by 12 selected and trained panellists from the Laboratorio Agroalimentario de Granada (Atarfe, Granada, Spain), according to the method described in Regulation EEC/796/2002 (EUC, 2002). The intensities of both the positive (fruity, bitter and pungent) and negative (fusty, winey, musty, muddy, rancid, metallic, and other) attributes were evaluated for each oil sample, on a non-structured, 10 cm scale, anchored by its origin.

Tocopherols were evaluated following the AOCS Method Ce 8–89 (AOCS, 1989). A solution of oil in hexane was analysed with HPLC (HP1100) on a silica gel Lichrosorb Si-60 column (particle size 5 μm, 250 × 4.6 mm i.d.; Sugerlabor, Madrid, Spain) which was eluted with hexane/2-propanol (95.5:1.5) at a flow rate of 1 ml/min. A fluorescence detector (Waters 470) with excitation and emission wavelengths set at 290 and 330 nm was used.

Total phenol compounds were isolated by extraction of a solution of oil in hexane, three times, with a water/methanol mixture (60:40). Folin–Ciocalteau reagent and sodium molybdate, 5% in 50% ethanol (Merck), were added to a suitable aliquot of the combined extracts and the absorbances of the solution at 725 nm were measured. Values were given as mg of caffeic acid per kg of oil (Gutfinger, 1981; Vázquez, Janer, & Janer, 1973).

Oxidative stability was evaluated by the Rancimat method (Gutiérrez, 1989). Stability was expressed as the oxidation induction time (hours), measured with the Rancimat 679 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 3.5 g warmed to 98 °C and air flow of 10 l/h.

In order to determine fatty acid composition (%), the methyl-esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium hydroxide solution, and analysed by GC with a Hewlett–Packard (HP 6890) chromatograph equipped with a FID Detector. A fused silica column (50 m length × 0.25 mm i.d.), coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor), was used. Helium was employed as a carrier gas with a flow through the column of 1 ml/min. The temperatures of the injector and detector were set at 250 °C with an oven temperature of 210 °C. An injection volume of 1 μl was used (Regulation EEC 2568/91, corresponding to AOCS method Ch 2–91).

Sterols (%) were determined with a Hewlett–Packard (HP 6890) gas chromatograph with a capillary column (25 m length × 0.25 mm i.d.) coated with SGL-5 (0.25 μm thickness; Sugerlabor). Working conditions were as follows: carrier gas, helium; flow through the column, 1.2 ml/min; injector temperature, 280 °C; detector temperature, 290 °C; oven temperature, 260 °C; injection volume 1 μl (Regulation EEC 2568/91, corresponding to AOCS method Ch 6–91). Apparent β-sitosterol was calculated as the sum of β-sitosterol, Δ5,23-stigmastadienol, clerosterol, sitostanol and Δ5,24-stigmastadienol.

Analytical tests were performed at least in duplicate.

2.1.5. Statistical analysis

Significant differences among varieties were determined by an analysis of variance which applied a Duncan test with a 95% significant level ($P < 0.05$), using the SPSS programme, release 11.5 for Windows.

2.2. Real quality

2.2.1. Sampling

The oil samples were taken directly from randomly selected stainless steel storage tanks at the oil mills. The first portions were discarded because of direct contact with the sampling tap. The samples were taken at the beginning (second fortnight of December, 2003) and at the end (first fortnight of February, 2004) of the harvest season, in each of the 20 oil mills situated in the study area. The total number of samples was 40 (20 oil mills × 2 collections).

2.2.2. Analytical determinations

The analytical determinations and the methodology followed are the same as those used to describe potential quality.

2.2.3. Statistical analysis

The mean values and standard deviation of the parameters analysed were determined using the SPSS programme, v. 11.5 for Windows.

3. Results and discussion

3.1. Potential quality

Table 1 shows the physicochemical and sensorial quality parameters for the olive oil samples from different varieties grown in the study area. All the oils produced and analysed showed very low values for the regulated physicochemical parameters evaluated (acidity $\leqslant 0.8°$; peroxide index $\leqslant$ 20 meq O_2/kg; $K_{270} \leqslant 0.22$; $K_{232} \leqslant 2.5$), with all of them falling within the ''extra virgin'' category, as stated by Regulation EC/1989/2003 (EUC, 2003). This is not surprising since the raw material was carefully selected, picked and processed. Note that lower values for these parameters will translate into a higher quality of oil. Although some significant differences in the values of free acidity and peroxide values were found between the varieties, the meanings of these differences were not clear. The ultraviolet absorbance (K_{270}, K_{232}), was not useful for discriminating among varieties.

As regards sensory quality, all the analysed samples were classified as ''extra virgin'', according to Regulation EEC/796/2000 (EUC, 2002), since the median of the defects was equal to 0 and the median of the fruity above 0. On the other hand, there were no significant differences among the varieties studied when comparing the median of the fruity attribute. The main differences among varieties lay in the intensities of the bitter and pungent positive attributes. *Picual* samples (53%) showed high intensities (above 5) for the bitter attribute, while 26% were above 5 for the pungent attribute, 21% were above 5 for both attributes and 42% were below that level for both attributes. *Cornicabra* samples (36%) showed high intensities (above 5) for the bitter attribute, 18% showed intensities above 5 for the pungent attribute, 13% showed intensities above 5 for both attributes and 59% showed intensities below 5 for both attributes. The pungent attribute exceeded level 5 in the variety *Manzanilla* in 25% of the samples. The *Arbequina* showed low values for both bitterness and pungency, remaining below 5 for all the samples. Last, the *Local* variety showed high values for the bitter attribute in all the samples, and for the pungent attribute in 50% of them.

The stability parameters are shown in Table 2. Only the total polyphenol content and the oxidative stability at 98 °C were useful for discriminating among varieties. The highest contents in total polyphenols were found in the varieties *Picual*, *Cornicabra* and *Local*. On the other hand, *Picual* and *Cornicabra*, were the most stable varieties over time; the *Local* variety, even though it had high total polyphenol content, was less stable, due to its lower tocopherol content. The oils from *Arbequina* and *Manzanilla* had low stability, but this negative property is currently mitigated by the small quantity of oil produced with respect to its demand, so the storage time is usually shorter than a year and the low stability does not imply a substantial quality loss. Nonetheless, this fact should be taken into account in a future scenario, with probably much higher productions than the current ones.

These results agreed with those reported by other authors studying the same varieties grown in other areas (Beltrán et al., 2000; Cert et al., 1999; Humanes & Civantos, 1993; Motilva et al., 1998; Salvador et al.,

Table 1
Means and standard deviations for the regulated physicochemical quality and sensory parameters evaluated in the olive oil samples analysed from the different olive varieties grown in the area studied

	Variety	Mean ± SD
Physicochemical quality parameters		
Free acidity (g/100 g)	*Picual*	0.15 ± 0.06^{bc}
	Cornicabra	0.19 ± 0.03^{ab}
	Manzanilla	0.16 ± 0.05^{bc}
	Arbequina	0.25 ± 0.07^{a}
	Local	0.10 ± 0.00^{c}
Peroxide value (meq/kg)	*Picual*	4.44 ± 1.16^{ab}
	Cornicabra	3.30 ± 0.83^{b}
	Manzanilla	4.54 ± 1.31^{ab}
	Arbequina	3.85 ± 2.53^{b}
	Local	5.70 ± 2.26^{a}
K_{270}	*Picual*	0.14 ± 0.23
	Cornicabra	0.13 ± 0.02
	Manzanilla	0.11 ± 0.01
	Arbequina	0.12 ± 0.03
	Local	0.14 ± 0.01
K_{232}	*Picual*	1.61 ± 0.14
	Cornicabra	1.56 ± 0.14
	Manzanilla	1.63 ± 0.12
	Arbequina	1.66 ± 0.07
	Local	1.73 ± 0.15
Sensory quality parameters		
Median of defects	*Picual*	0
	Cornicabra	0
	Manzanilla	0
	Arbequina	0
	Local	0
Median of fruity	*Picual*	4.68 ± 0.71
	Cornicabra	4.81 ± 0.72
	Manzanilla	5.48 ± 0.92
	Arbequina	5.30 ± 0.14
	Local	5.80 ± 0.42
Panel test classification	*Picual*	Extra Virgin
	Cornicabra	Extra Virgin
	Manzanilla	Extra Virgin
	Arbequina	Extra Virgin
	Local	Extra Virgin

[a,b,c] Different superscripts for the same quality parameter mean significant differences among varieties.

Table 2
Means and standard deviations for the stability parameters evaluated in the olive oil samples analysed from the different olive varieties grown in the studied area

Stability parameters	Variety	Mean ± SD
Tocopherols (mg/kg)	*Picual*	190 ± 40.7
	Cornicabra	206 ± 80.9
	Manzanilla	210 ± 29.8
	Arbequina	250 ± 87.8
	Local	172 ± 23.2
Total phenols (mg/kg)	*Picual*	652 ± 109[a]
	Cornicabra	555 ± 176[a]
	Manzanilla	322 ± 117[b]
	Arbequina	244 ± 108[b]
	Local	575 ± 87[a]
Oxidative stability at 98 °C (h)	*Picual*	157 ± 21.3[a]
	Cornicabra	130 ± 19.4[a]
	Manzanilla	60.1 ± 14.4[c]
	Arbequina	51.3 ± 25.7[c]
	Local	95.6 ± 13[b]

[a,b,c] Different superscripts for the same quality parameter mean significant differences among varieties.

2001, Salvador, Aranda, Gómez-Alonso, & Fregapane, 2003; Tous et al., 1997; Uceda, Aguilera, Beltrán, & Jiménez, 2000), and the values were within the usual range for virgin olive oil (Vázquez et al., 1973).

The distribution of fatty acid composition (Table 3) covered the normal range expected for olive oil (Humanes & Civantos, 1993; Alba et al., 1996; Tous et al., 1997; Ranelli, Mattia, Ferrante, & Giansante, 1997; Motilva et al., 2001; Salvador et al., 2001, 2003). The highest content of palmitic acid was found in *Arbequina*, followed by *Manzanilla*. This situation was reversed for the stearic acid content. The palmitoleic and margaroleic acid contents were also significantly higher in the variety *Arbequina* than in the others. The variety *Manzanilla* and *Local* varieties showed the highest linoleic values.

The oleic and linoleic acid contents were the most useful and significant parameters for differentiating the predominant varieties, *Picual* and *Cornicabra*, from the others. Both varieties showed high oleic values and low linoleic values, unlike the others. *Cornicabra* and *Picual* are the Spanish varieties with the lowest linoleic acid levels (Alba et al., 1996). The low oleic and high linoleic acid contents shown by *Arbequina* and *Manzanilla* seemed to contribute to their low oxidative stability (Table 2), since this leads to a low mono/polyunsaturated ratio (Beltrán et al., 2000). Previous works (Civantos, Contreras, & Grana, 1992; Tous et al., 1997) have shown that, when the *Arbequina* variety is grown farther south, the proportion of saturated fatty acids is higher in its oils, the content of oleic acid decreases and the content of linoleic acid increases. The results of this work concur with this for oleic acid, where our values were lower than those found in Lleida and Tarragona oils (north-eastern Spain), and higher than those found in Córdoba (southern Spain), but differ with regard to the linoleic acid, since our results were similar to those found in north-eastern Spain. The content of arachidic and, mainly, gadoleic acid, was very useful for discriminating between the predominant varieties, *Picual* and *Cornicabra*, the latter having the higher values in both cases. No significant differences were found among varieties for the rest of the fatty acids evaluated (margaric, behenic, lignoceric) or for the *trans*-oleic isomers content.

Sterol and triterpenic dialcohol composition is shown in Table 4. β-Sitosterol content in the *Arbequina* variety was significantly lower than in the other varieties. On the other hand, the apparent β-sitosterol content, which also takes into account some sterols formed by the degradation of β-sitosterol, was not useful for discriminating among varieties. The Δ5-avenasterol and Δ7-stigmastenol contents were significantly higher in *Arbequina*, which disagreed with that published by other researchers (Alba et al., 1996), probably because the oils came from very young trees (3–4 years) which still had not reached full agronomic development. The antioxidant activity of Δ5-avenasterol has also been pointed out by some works (Williamson, 1988), but our results seem to disagree with this, as

Table 3
Means and standard deviations for the fatty acids composition (%) in the olive oil samples analysed from the different olive varieties grown in the studied area

Variety	Palmitic C16:0	Palmitoleic C16:1	Margaric C17:0	Margaroleic C17:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2
Picual	10.5 ± 0.66[c]	0.78 ± 0.12[bc]	<0.10	0.10 ± 0.00[c]	2.93 ± 0.29[a]	80.7 ± 1.21[a]	3.51 ± 0.85[c]
Cornicabra	10 ± 0.89[c]	0.82 ± 0.14[bc]	<0.10	0.10 ± 0.00[c]	3.32 ± 0.43[a]	80.0 ± 0.74[a]	4.09 ± 0.61[c]
Manzanilla	13.2 ± 0.90[b]	0.94 ± 0.37[b]	<0.10	0.10 ± 0.00[c]	2.30 ± 0.34[b]	73.5 ± 3.30[c]	8.34 ± 1.77[ab]
Arbequina	14.9 ± 0.50[a]	2.00 ± 0.14[a]	0.10	0.25 ± 0.07[a]	1.70 ± 0.00[c]	70.6 ± 0.78[d]	9.05 ± 0.35[a]
Local	10.0 ± 1.13[c]	0.65 ± 0.07[c]	0.10	0.20 ± 0.00[b]	2.95 ± 0.00[a]	77 ± 0.92[b]	7.60 ± 0.00[b]

Variety	Linolenic C18:3	Arachidic C20:0	Gadoleic C20:1	Behenic C22:0	Lignoceric C24:0	*trans*-oleic isomers C18:1 T
Picual	0.62 ± 0.07[c]	0.39 ± 0.03[b]	0.20 ± 0.00[c]	0.10 ± 0.00	0.06 ± 0.02	<0.10
Cornicabra	0.66 ± 0.08[c]	0.50 ± 0.04[a]	0.30 ± 0.00[a]	0.14 ± 0.05	0.09 ± 0.03	<0.10
Manzanilla	0.84 ± 0.09[a]	0.40 ± 0.00[b]	0.26 ± 0.05[b]	0.10 ± 0.00	0.09 ± 0.03	<0.10
Arbequina	0.70 ± 0.00[bc]	0.40 ± 0.00[b]	0.30 ± 0.00[a]	0.10 ± 0.00	0.10 ± 0.00	<0.10
Local	0.80 ± 0.00[ab]	0.35 ± 0.07[b]	0.25 ± 0.07[b]	0.10 ± 0.00	0.05 ± 0.02	<0.10

[a,b,c] Different superscripts for the same quality parameter mean significant differences among varieties.

Table 4
Means and standard deviations for the sterol and triterpenic dialcohols (eritrodiol + uvaol) composition (%) in the olive oil samples from the different olive varieties grown in the studied area

Variety	24-Methylenecholesterol	Campesterol	Campestanol	Stigmasterol	Δ7-Campesterol	Δ5,23-Stigmastadienol	Clerosterol	β-Sitosterol
Picual	0.14 ± 0.07^{c}	3.25 ± 0.14^{b}	0.15 ± 0.07	0.44 ± 0.14^{b}	<0.10	0.10 ± 0.00	0.85 ± 0.08^{ab}	85.8 ± 2.28^{a}
Cornicabra	0.14 ± 0.06^{c}	3.81 ± 0.22^{a}	0.11 ± 0.02	0.47 ± 0.10^{b}	<0.10	0.10 ± 0.00	0.91 ± 0.06^{ab}	87 ± 2.41^{a}
Manzanilla	0.38 ± 0.17^{b}	3.00 ± 0.26^{b}	0.12 ± 0.04	0.72 ± 0.33^{a}	<0.10	0.10 ± 0.00	0.86 ± 0.09^{ab}	85.7 ± 1.08^{a}
Arbequina	0.65 ± 0.21^{a}	2.65 ± 0.35^{c}	0.10 ± 0.00	0.65 ± 0.07^{ab}	0.10	0.10 ± 0.00	0.80 ± 0.00^{b}	82.5 ± 2.67^{b}
Local	0.10 ± 0.00^{c}	2.70 ± 0.14^{c}	0.10 ± 0.00	0.50 ± 0.00^{ab}	<0.10	0.10 ± 0.00	0.95 ± 0.07^{a}	85.7 ± 1.62^{a}

Variety	Sitostanol	Δ5-Avenasterol	Δ5,24-Stigmastadienol	Δ7-Stigmastenol	Δ7-Avenasterol	Apparent β-Sitosterol	Total sterols (mg/kg)	Erythrodiol + Uvaol
Picual	0.80 ± 0.26^{a}	7.59 ± 2.10^{b}	0.34 ± 0.10^{b}	0.26 ± 0.10^{b}	0.41 ± 0.18^{a}	95.3 ± 0.16	1053 ± 100^{c}	1.65 ± 0.42^{b}
Cornicabra	0.42 ± 0.07^{b}	6.24 ± 2.22^{b}	0.40 ± 0.16^{b}	0.15 ± 0.06^{b}	0.29 ± 0.07^{ab}	94.6 ± 2.14	1508 ± 132^{b}	3.70 ± 0.83^{a}
Manzanilla	0.34 ± 0.09^{b}	7.88 ± 1.27^{b}	0.50 ± 0.10^{ab}	0.14 ± 0.05^{b}	0.34 ± 0.09^{ab}	95.3 ± 0.49	1315 ± 339^{b}	2.24 ± 0.71^{b}
Arbequina	0.45 ± 0.21^{b}	10.9 ± 3.15^{a}	0.65 ± 0.21^{a}	0.40 ± 0.17^{a}	0.20 ± 0.14^{b}	95.4 ± 0.48	1735 ± 301^{a}	1.75 ± 0.35^{b}
Local	0.40 ± 0.00^{b}	8.50 ± 1.84^{b}	0.40 ± 0.00^{b}	0.10 ± 0.00^{b}	0.45 ± 0.07^{a}	96.0 ± 0.07	1274 ± 11.3^{bc}	3.75 ± 0.92^{a}

[a,b,c] Different superscripts for the same quality parameter mean significant differences among varieties.

Arbequina was one of the least stable varieties (Table 2). As regards the 24-methylenecholesterol content, the variety *Arbequina* stood out, followed by *Manzanilla*. The campesterol content was very high in *Cornicabra*, although lower than the values reported by some authors (Aranda, 2003; Salvador et al., 1998, 2001, 2003), who found values exceeding the maximum allowed level (4%) in the regulation EC/1989/2003 (EUC, 2003); while *Arbequina* and *Local* were the varieties with the lowest contents. The stigmasterol content for *Manzanilla* was significantly higher than the values for *Picual* and *Cornicabra*. The campestanol, Δ7-campesterol and Δ5,23-stigmastadienol contents were not useful for discriminating among varieties. The clerosterol content was useful only to discriminate between the *Arbequina* and *Local* varieties. The highest sitostanol content was found in *Picual*. The Δ5,24-stigmastadienol content for the *Arbequina* variety was significantly higher than the values for the other varieties, except for *Manzanilla*. The Δ7-avenasterol for *Arbequina* was significantly lower than the values for *Local* and *Picual*. *Arbequina* showed the highest values for the total sterol content, while *Local* and Picual had the lowest. For all the cases, the minimum 1000 mg/kg allowed by Regulation EC/1989/2003 (EUC, 2003) was exceeded. The varieties *Local* and *Cornicabra* stood out in the triterpenic dialcohols (erythrodiol + uvaol), with values significantly different from the other varieties. The most useful parameters for discriminating among the predominant varieties, *Picual* and *Cornicabra*, as regards the sterol and triterpenic dialcohol composition, were campesterol, sitostanol, total sterol and erythrodiol + uvaol. Note that *Cornicabra* had higher campesterol, erythrodiol + uvaol and total sterol contents, while *Picual* had a higher sitostanol content.

3.2. Real quality

The composition parameters evaluated in the olive oil samples from the different oil mills in the study area are shown in Table 5. All the analysed oils showed very low values for the regulated physicochemical quality parameters. Therefore, all of them qualified for the "extra virgin" category, according to Regulation EC/1989/2003 (EUC, 2003). These values confirmed the high quality of the olive oils from the studied area.

The predominance of the varieties, *Picual* and *Cornicabra*, was the main cause for the high stability found in the oils analysed. These values guarantee a high commercial price for the virgin olive oils from the area. The fatty acid, sterol and triterpenic dialcohol compositions complied with all the requirements of Regulation EC/1989/2003 (EUC, 2003).

The fatty acid composition of the analysed olive oils showed intermediate values between those observed for the *Picual* and *Cornicabra* varieties in the potential quality study (Table 3). The oils of the studied area stood out for their high contents of oleic and stearic acids, and for their low contents of linoleic, palmitic and linolenic acids.

Table 5
Means and standard deviations for the regulated physicochemical quality parameters, stability, fatty acids, sterols and triterpenic dialcohols (erythrodiol + uvaol) composition evaluated in the olive oil samples from the different oil mills of the studied area

	Mean ± SD
Physicochemical quality parameters	
Free acidity (g/100 g)	0.22 ± 0.13
Peroxide value (meq/kg)	4.83 ± 1.02
K_{270}	0.15 ± 0.02
K_{232}	1.71 ± 0.08
Stability parameters	
Tocopherols (mg/kg)	178 ± 35.2
Total phenols (ppm)	609 ± 69.9
Oxidative stability at 98 °C (h)	163 ± 32.6
Fatty acids (%)	
Palmitic C16:0	10.2 ± 0.55
Palmitoleic C16:1	0.75 ± 0.06
Margaric C17:0	<0.10
Margaroleic C17:1	0.10 ± 0.00
Stearic C18:0	3.15 ± 0.23
Oleic C18:1	80.7 ± 0.53
Linoleic C18:2	3.69 ± 0.51
Linolenic C18:3	0.61 ± 0.04
Arachidic C20:0	0.42 ± 0.04
Gadoleic C20:1	0.23 ± 0.05
Behenic C22:0	0.11 ± 0.02
Lignoceric C24:0	<0.10
trans-oleic isomers C18:1 T	<0.05
Sterols (%)	
24-Methylenecholesterol	0.15 ± 0.06
Campesterol	3.41 ± 0.22
Campestanol	0.15 ± 0.06
Stigmasterol	0.55 ± 0.14
Δ7-Campesterol	<0.10
Δ5,23-Stigmastadienol	<0.10
Clerosterol	0.85 ± 0.06
β-Sitosterol	86.3 ± 0.86
Sitostanol	0.72 ± 0.16
Δ5-Avenasterol	6.85 ± 1.05
Δ5,24-Stigmastadienol	0.39 ± 0.10
Δ7-Stigmastenol	0.23 ± 0.07
Δ7-Avenasterol	0.30 ± 0.06
Apparent β-Sitosterol	95.0 ± 1.56
Total sterols (mg/kg)	1208 ± 116
Erythrodiol + Uvaol	2.21 ± 0.71

The sterol composition was also intermediate between those found for *Picual* and *Cornicabra* in the potential quality study (Table 4). The oils stood out for their high β-sitosterol content and for their low contents of 24-methylenecholesterol, stigmasterol and Δ5-avenasterol. The contents of campesterol, erythrodiol + uvaol, and total sterols varied according to the predominance of one or the other variety, with higher values for these parameters occurring when the proportion of *Cornicabra* olives was higher, and vice versa.

The sensory analysis yielded 69% of the analysed samples as classified within the "extra virgin" category, while the other 31% fell within the "virgin" category (the median of the defects was above 0 and below or equal to 2.5 and the median of fruity above 0), according to Regulation EEC/796/2000 (EUC, 2002). Analysed samples (48%) showed high bitter intensities (above 5); 24% exceeded intensity 5 for the pungent attribute, while 50% of these did not exceed such intensity for either of these attributes. 22% of the samples showed high intensities (above 5) for both bitter and pungent attributes.

In conclusion, the main characteristic of the oils from the future DO "Campo de Montiel" were as follows: the high proportion of the *Picual* and *Cornicabra* varieties gave its oil remarkably high stability (due to their high tocopherol and total polyphenol contents), as well as a high oleic acid content and low linoleic acid content, where the *Cornicabra* variety was predominant, the oils had high campesterol content.

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