Quality and Composition of Virgin Olive Oil from Varieties Grown in Castilla-La Mancha (Spain)

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Abstract: The regulated physicochemical quality parameters (free acidity, peroxide value and UV absorption characteristics), sensory parameters (median of fruity, median of defect, panel classification, bitterness and pungency), stability parameters (total phenols and oxidative stability at 100°C) and chemical composition (fatty acids, sterols and triterpenic dialcohols) of virgin olive oils obtained from 12 olive varieties cultivated in 6 of the most representative zones of Castilla-La Mancha (La Alcarria, Campos de Calatrava, Campos de Hellín, Campos de Montiel, Montes de Toledo and Sierra de Alcaraz) were evaluated. The varieties Cornicabra and Picual showed remarkable total polyphenols content and high stability, in contrast with Arbequina. The other less common varieties were in-between these two groups. Cornicabra and Picual showed also high oleic and low linoleic acids content, while Arbequina showed low oleic, high linoleic high palmitic and high palmitoleic acid content. The varieties Benizal and Cornicabra showed very high campesterol content. Benizal stood out by its high stigmasterol, low apparent β-sitosterol and low total sterols content, and the latter was below the established limit for olive oil. Triterpenic dialcohol content was significantly lower for Arbequina than for Cornicabra.

Key words: virgin olive oil, quality, sensory, variety, origin

1 Introduction
The olive trees (Olea europaea) are cultivated mainly in the Mediterranean Basin. EU countries account for 70-80% of the world production. Italy and Spain are the two main producing countries and together they represent about 75% of the total surface and production1. Spain had in 2008 a surface of olive plantations of about 2.5·10^6 ha. Castilla-La Mancha had about 350·10^3 ha, being the second most important region after Andalusia. Most of the olive plantation surface in Castilla-La Mancha is concentrated in two Provinces: Ciudad Real and Toledo, which sum about 70% of the total surface.

Most of the olive plots in Castilla-La Mancha are located in marginal areas, subject to harsher climatic conditions than the Andalusian olive groves and yielding a much lower production. Thus, although the area planted in Castilla-La Mancha accounts for 14% of Spain’s olive surface, its oil production accounts for only 6-7% of national production. Therefore, characterization and differentiation of the oil produced in this region is vital to compete in an increasingly global market.

The most characteristic and common variety in Castilla-La Mancha is Cornicabra. The Picual variety predominates in the South of the region on the border with Andalusia. Recently, intensive irrigated plantations of the variety Arbequina, whose oil is prized for its sensory properties, have been set up. The quality and characteristics of the variety Cornicabra in Castilla-La Mancha have been thoroughly studied2–6. Papers have also been published on the quality of oil produced in certain areas of Castilla-La Mancha: Campo de Montiel6, Montes de Alcaraz7 and Campos de Hellín8. However, a study that characterizes the olive oil produced in the region of Castilla-La Mancha, including the main varieties of olives grown in the region, has not been done.

Virgin olive oil is obtained by exclusively physical extraction methods (pressure, centrifugation or sedimentation). Traditionally, its characterization has been performed based on the regulated analytical parameters that are related to a possible state of deterioration, rather than positive intrinsic qualities. Regulated physicochemical parameters are required by law for the commercial classification of olive oils, but do not take into account the special properties of these oils and their advantages compared to other
vegetable fats.

Oil stability determines the product shelf life and is related to its content in phenolic compounds, which are natural antioxidants present in olive oils and are shown to have beneficial properties on human health.\textsuperscript{9, 10}

Fatty acid composition is highly dependent on genetic and environmental factors and has been previously used for classification of virgin olive oils\textsuperscript{11-15}.

The composition of the sterol fraction is a very useful parameter to detect adulteration, since it can be considered a real fingerprint of olive oil\textsuperscript{16}. It has been found that some varieties, such as Cornicabra sometimes exceed the upper limits set for campesterol in virgin olive oil\textsuperscript{2, 3, 5, 17}. The percentage of triterpenic dialcohols (erythrodiol and uvaol) is also limited in virgin olive oil\textsuperscript{18}.

The objective of this work was to characterize the olive oils from Castilla-La Mancha region and determine the influence of the variety on the regulated quality parameters, stability and chemical composition.

2 Material and methods

2.1 Selection of plots and trees within the studied zone

A total of 128 plots were selected in 6 different zones, planted with 12 different varieties (Table 1). Within each plot, 6 typical trees of 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 (Andalucía, España).

2.2 Sampling

Samples were taken at two precise moments: (i) the beginning of the harvest season (first fortnight of December 2010), 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 2011), when all the fruits were ripe, some of them even over-ripe. In both cases the sampling was begun by the earlier varieties. The total number of samples was 256 (128 plots × 2 collections). The results of the first and second sampling were combined for the statistical analysis.

In each plot, 15 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), Albacete.

2.3 Olive oil extraction

An experimental oil mill comprising a hammer mill, malaxator and decanter (Oliomio TF-30, Toscana Enológica Mori, Tavarnelle Val di Pesa - FI-Italia) was used. The oil mill worked in the 2-phase system. About 2 L of oil was obtained from each olive sample. The oil was decanted and placed in small bottles for further analysis.

2.4 Analytical determinations

Determination of the regulated physicochemical quality parameters (free acidity, peroxide value and UV absorption characteristic, K\textsubscript{270} and K\textsubscript{232}) was carried out following the

| Zone                    | Variety          | Sampled plots |
|------------------------|------------------|---------------|
| Campo de Calatrava     | Arbequina        | 14            |
|                        | Cornicabra       | 8             |
|                        | Picual           | 5             |
| Campo de Montiel       | Arbequina        | 29            |
|                        | Cornicabra       | 2             |
|                        | Manzanilla de Centro | 10         |
|                        | Manzanilla Local | 2             |
|                        | Picual           | 12            |
| Campos de Hellín       | Arbequina        | 36            |
|                        | Benizal          | 6             |
|                        | Cornicabra       | 4             |
|                        | Cuquillo         | 6             |
|                        | Injerta          | 2             |
|                        | Manzanilla de Sevilla | 2        |
|                        | Manzanilla Local | 2             |
|                        | Negrilla         | 2             |
|                        | Picual           | 8             |
| La Alcarria            | Castellana       | 12            |
| Montes de Toledo       | Arbequina        | 16            |
|                        | Cornicabra       | 1             |
|                        | Picual           | 14            |
|                        |          | 1             |
| Sierra de Alcaraz      | Cornicabra       | 21            |
|                        | Manzanilla de Centro | 6         |
|                        | Manzanilla Local | 2             |
|                        | Onil             | 2             |
|                        | Picual           | 9             |
Virgin olive oil quality

analytical methods described by Regulation EU 1348/2013 of the Commission of the European Union²⁰.

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

Peroxide value, expressed in milliequivalents of active oxygen per kilogram 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.

K₂₇₀ and K₂₃₂ extinction coefficients were calculated from absorption at 270 and 232 nm, respectively, with an UV spectrophotometer (Hewlett-Packard, HP 8452A), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Sensory analysis (median of fruity, median of defects, panel classification, bitterness and pungency) of the samples was carried out by 12 selected and trained panelists from the panel of Laboratorio Agroalimentario de Granada (Atarfe, Granada, Spain), according to the method described by Regulation EU 1348/2013²⁰. The intensity of bitterness and pungency was evaluated as the percentage of samples with the attribute value higher than 5 on the 10-cm scale shown in the profile sheet provided.

Total phenols compounds were determined by Folin-Ciocalteu colorimetric method. A solution of 10 g of oil in 75 mL of hexane was extracted with a water/methanol mixture (60:40), three times and completed to 100 mL with distilled deionized water. A 2 mL aliquot of the methanolic extract was mixed with 1 mL Folin-Ciocalteu reagent (Merck), 2 mL saturated sodium carbonate and 15 mL of distilled water and the absorptions of the solution at 725 nm were measured. Values were given as mg of caffeic acid per kg of oil²⁰.

Oxidative stability was evaluated by the Rancimat method²¹. 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 100°C and air flow of 10 L/h.

For the determination of 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 potash, and analysed by GC 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), as explained elsewhere²¹. 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 EC 2568/91, corresponding to AOCS method Ch 6-91). Apparent β-sitosterol included the sum of β-sitosterol, Δ5,23-stigmastadienol, clerosterol, sitostanol and Δ5,24-stigmastadienol.

Analytical determinations were carried out at least in duplicate.

2.5 Statistical analysis

Significant differences among varieties were determined by an analysis of variance applying a Tukey test with a 95% significant level (p<0.05), using the SPSS programme for Windows.

3 Results and discussion

Table 2 shows the physicochemical and sensory quality parameters for the olive oil samples from different varieties grown in the studied area. All the produced and analysed oils showed very low values for the regulated physical-chemical parameters evaluated (acidity ≤ 0.8%, peroxide index ≤ 20 meq O₂/kg, K₂₇₀ ≤ 2.5, K₂₃₂ ≤ 0.22), meaning high olive oil quality. This result was expected, because the olive samples were carefully picked, and the oil was extracted under optimal conditions. Therefore, all the olive samples were classified as “extra virgin” category, as stated by Regulation EU 1348/2013²⁰. The variety Cuquillo gave the highest values of free acidity, significantly different from Arbequina, Benizal, Castellana and Negrilla. Cuquillo showed also the highest peroxide values, but only significantly different from Cornicabra, Onil and Picual. There were not significant differences in the ultraviolet absorbance parameters (K₂₃₂, K₂₇₀) between varieties.

Table 2 shows the sample average of the median of fruity for each variety. Fruity was always higher than 0 and there were not significant differences in this attribute. The median of defects was equal to zero (data not shown in Table 2), and therefore all the samples were classified as extra virgin oil²⁰.

The varieties Arbequina and Negrilla had similar characteristics as regards bitterness and pungency. All the samples of both varieties had values less than 5 for these positive attributes. Note that the phenols content and oxidative stability of Negrilla were lower but not significantly different than those of Manzanilla Local, while the bitterness and pungency of the former were strongly higher than the latter. The reason for this apparent inconsistency could be that the number of samples of these minor varieties was
Local samples having bitterness values above 5 was Manzanilla content of these varieties and Picual could also be due to demonstrate the existence of significant differences. The higher number of samples could have been necessary to very low (2 and 6 respectively, as shown in Table 1), and a higher number of samples could have been necessary to demonstrate the existence of significant differences. The lack of significant differences between the total phenols content of these varieties and Picual could also be due to this reason. The variety with the highest percentage of samples having bitterness values above 5 was Manzanilla Local (100%), followed by Onil (75%), Cornicabra (68.2%) and Picual (64.3%). The variety with the highest percentage of samples having pungency values above 5 was Benizal (87.5%).

The stability parameters, total phenols content and oxidative stability at 98°C, were both useful for discriminating among varieties (Table 3). Picual had the highest phenols content, but only significantly different from Arbequina and Manzanilla de Sevilla. Picual, together with Cornicabra, showed the highest values of oxidative stability. Note that generally the lowest stability values were found for the varieties with the lowest phenols contents and that they coincided with the varieties showing the lowest bitter-

Table 2  Regulated physicochemical and sensory parameters for the different olive varieties.

| Variety (n)               | Free acidity (g/100 g) | Peroxide value (meq/kg) | K_{232} | K_{120} | Fruity (0-10) | Bitterness (%) | Pungency (%) |
|---------------------------|------------------------|-------------------------|---------|---------|---------------|----------------|--------------|
| Arbequina (10)            | 0.13 ± 0.05<sup>a</sup> | 6.3 ± 2.1<sup>b</sup> | 1.688 ± 0.158 | 0.092 ± 0.016 | 4.96 ± 0.56 | 0 | 0 |
| Benizal (4)               | 0.13 ± 0.05<sup>b</sup> | 5.5 ± 1.2<sup>ab</sup> | 1.658 ± 0.096 | 0.124 ± 0.012 | 5.40 ± 0.57 | 37.5 | 87.5 |
| Castellana (12)           | 0.10 ± 0.00<sup>a</sup> | 6.0 ± 0.8<sup>ab</sup> | 1.710 ± 0.088 | 0.105 ± 0.006 | 3.57 ± 1.08 | 25 | 75 |
| Cornicabra (44)           | 0.18 ± 0.07<sup>abc</sup> | 4.4 ± 1.9<sup>bc</sup> | 1.578 ± 0.164 | 0.118 ± 0.029 | 6.21 ± 4.48 | 68.2 | 75 |
| Cuquillo (4)              | 0.23 ± 0.05<sup>c</sup> | 7.7 ± 2.2<sup>bc</sup> | 1.657 ± 0.139 | 0.114 ± 0.029 | 5.46 ± 0.75 | 50 | 75 |
| Injerta (2)               | 0.13 ± 0.05<sup>abc</sup> | 5.7 ± 1.2<sup>ab</sup> | 1.606 ± 0.053 | 0.103 ± 0.019 | 5.61 ± 1.16 | 25 | 50 |
| Manzanilla de Centro (5)  | 0.15 ± 0.05<sup>abc</sup> | 4.7 ± 1.3<sup>ab</sup> | 1.607 ± 0.114 | 0.105 ± 0.012 | 6.16 ± 0.57 | 40 | 30 |
| Manzanilla de Sevilla (2) | 0.21 ± 0.11<sup>b</sup> | 6.7 ± 1.2<sup>ab</sup> | 1.616 ± 0.138 | 0.101 ± 0.019 | 5.77 ± 0.46 | 25 | 75 |
| Manzanilla Local (6)      | 0.16 ± 0.05<sup>abc</sup> | 5.0 ± 1.3<sup>ab</sup> | 1.689 ± 0.107 | 0.129 ± 0.013 | 5.16 ± 1.26 | 100 | 58.3 |
| Negrilla (2)              | 0.10 ± 0.00<sup>a</sup> | 6.1 ± 0.7<sup>ab</sup> | 1.753 ± 0.049 | 0.103 ± 0.025 | 5.40 ± 0.53 | 0 | 0 |
| Onil (2)                  | 0.13 ± 0.06<sup>c</sup> | 4.2 ± 3.0<sup>bc</sup> | 1.563 ± 0.127 | 0.097 ± 0.006 | 4.50 ± 1.32 | 75 | 75 |
| Picual (35)               | 0.15 ± 0.06<sup>abc</sup> | 4.3 ± 2.0<sup>c</sup> | 1.552 ± 0.133 | 0.121 ± 0.031 | 5.31 ± 1.07 | 64.3 | 52.9 |

n: number of samples; <sup>a</sup>, <sup>b</sup>, <sup>c</sup>: Different superscripts for the same quality parameter mean significant differences among varieties;

(1) Mean ± SD (2): Mean of median of fruity ± SD; (3): percentage of samples with sensory parameters higher than 5.

Table 3  Stability parameters (mean±SD) for the different olive varieties.

| Variety (n) | Total phenols (mg/kg) | Oxidative stability at 100°C (h) |
|-------------|-----------------------|----------------------------------|
| Arbequina (10) | 242 ± 69<sup>a</sup> | 40.6 ± 8.9<sup>a</sup> |
| Benizal (4)   | 489 ± 111<sup>abc</sup> | 49.7 ± 10.0<sup>a</sup> |
| Castellana (12) | 391 ± 34<sup>abc</sup> | 53.2 ± 2.0<sup>a</sup> |
| Cornicabra (44) | 556 ± 214<sup>bc</sup> | 108.4 ± 37.2<sup>bc</sup> |
| Cuquillo (4)  | 417 ± 112<sup>abc</sup> | 64.3 ± 10.5<sup>ab</sup> |
| Injerta (2)   | 365 ± 64<sup>abc</sup> | 50.5 ± 4.8<sup>a</sup> |
| Manzanilla de Centro (5) | 384 ± 106<sup>abc</sup> | 67.8 ± 15.7<sup>ab</sup> |
| Manzanilla de Sevilla (2) | 301 ± 66<sup>ab</sup> | 45.1 ± 5.5<sup>a</sup> |
| Manzanilla Local (6) | 523 ± 56<sup>bc</sup> | 78.4 ± 5.2<sup>ab</sup> |
| Negrilla (2)  | 391 ± 61<sup>abc</sup> | 36.6 ± 5.4<sup>a</sup> |
| Onil (2)      | 400 ± 86<sup>abc</sup> | 79.6 ± 11.5<sup>ab</sup> |
| Picual (35)   | 605 ± 180<sup>c</sup> | 130.6 ± 41.2<sup>c</sup> |

n: number of samples; a, b, c: Different superscripts for the same quality parameter mean significant differences among varieties.
Table 4 Mean and standard deviation for the fatty acids composition (%) for the different olive varieties.

| Variety (n)       | Palmitic C16:0 | Palmitoleic C16:1 | Margaric C17:0 | Margaroleic C17:1 | Stearic C18:0 | Oleic C18:1 |
|-------------------|----------------|-------------------|---------------|-------------------|--------------|------------|
| Arbequina (10)    | 14.40 ± 0.98a  | 1.70 ± 0.37d      | <0.10b        | 0.23 ± 0.05c      | 1.70 ± 0.09a | 70.24 ± 1.84c|
| Benical (4)       | 12.14 ± 0.59ad | 0.82 ± 0.14bc     | 0.11 ± 0.03g  | 0.12 ± 0.06g      | 2.00 ± 0.32ab| 76.67 ± 1.35ad|
| Castellana (12)   | 12.85 ± 0.25f  | 1.00 ± 0.00c      | <0.10b        | <0.10b            | 2.70 ± 0.08ef| 76.35 ± 0.66ef|
| Cornicabra (44)   | 10.06 ± 0.91ab | 0.81 ± 0.16abc    | <0.10b        | <0.10b            | 3.11 ± 0.38fg| 80.61 ± 1.19fg|
| Cuquillo (4)      | 11.36 ± 0.67bc | 0.66 ± 0.10abc    | <0.10b        | <0.10b            | 3.34 ± 0.25fg| 76.33 ± 1.51bc|
| Injerta (2)       | 10.94 ± 0.64ef | 0.59 ± 0.04d      | <0.10b        | <0.10b            | 1.99 ± 0.04ef| 78.81 ± 0.70ef|
| Manzanilla de Centro (5) | 12.08 ± 0.59d | 0.70 ± 0.07b      | <0.10b        | <0.10b            | 2.23 ± 0.17ef| 75.81 ± 1.28ef|
| Manzanilla de Sevilla (2) | 10.67 ± 0.38c | 0.68 ± 0.07b      | <0.10b        | <0.10b            | 2.76 ± 0.35ef| 76.64 ± 1.07ed|
| Manzanilla Local (6) | 9.77 ± 0.70d | 0.66 ± 0.08a      | 0.11 ± 0.04c  | 0.23 ± 0.05d      | 3.07 ± 0.41ef| 77.43 ± 0.68ed|
| Negrilla (2)      | 14.57 ± 0.59b  | 1.57 ± 0.15c      | <0.10b        | <0.10b            | 2.57 ± 0.38ef| 72.17 ± 0.70ef|
| Onil (2)          | 12.20 ± 0.30cd | 1.07 ± 0.15c      | 0.17 ± 0.06c  | 0.33 ± 0.06c      | 2.47 ± 0.15ef| 74.70 ± 0.85ef|
| Picual (35)       | 10.68 ± 0.85bc | 0.82 ± 0.20abc    | <0.10b        | <0.10b            | 2.93 ± 0.31deg| 80.77 ± 1.31ef|

Table 2

Linoleic C18:2 | Linoleic C18:3 | Arachidic C20:0 | Gadoleic C20:1 | Behenic C22:0 | Lignoceric C24:0 |
|---------------|---------------|----------------|---------------|--------------|-----------------|
| Arbequina (10) | 10.19 ± 1.00a | 0.58 ± 0.09ab  | 0.40 ± 0.00bc | 0.30 ± 0.00ad | <0.10           | <0.10          |
| Benical (4)    | 6.54 ± 1.32c  | 0.82 ± 0.11a    | 0.38 ± 0.06bc | 0.34 ± 0.05ac | <0.10           | <0.10          |
| Castellana (12) | 5.42 ± 0.39a  | 0.72 ± 0.05c    | 0.40 ± 0.00ac | 0.25 ± 0.06bc | <0.10           |
| Cornicabra (44) | 3.58 ± 0.62a  | 0.61 ± 0.09ab   | 0.49 ± 0.04c  | 0.30 ± 0.02ad | 0.12 ± 0.04     |
| Cuquillo (4)   | 6.58 ± 0.97ac | 0.69 ± 0.11bc   | 0.46 ± 0.05cd | 0.23 ± 0.04e   | <0.10           |
| Injerta (2)    | 6.39 ± 0.29ac | 0.80 ± 0.10c    | 0.37 ± 0.05bc | 0.37 ± 0.05f   | <0.10           |
| Manzanilla de Centro (5) | 7.62 ± 0.97ad | 0.71 ± 0.08ac   | 0.35 ± 0.05c  | 0.29 ± 0.03ce  | <0.10           |
| Manzanilla de Sevilla (2) | 7.67 ± 0.97ad | 0.82 ± 0.08c    | 0.46 ± 0.05cd | 0.36 ± 0.05ef  | <0.10           |
| Manzanilla Local (6) | 7.27 ± 0.72ad | 0.79 ± 0.04c    | 0.43 ± 0.08bd | 0.29 ± 0.04e   | <0.10           |
| Negrilla (2)   | 7.30 ± 0.52ad | 0.83 ± 0.06d    | 0.40 ± 0.10ad | 0.30 ± 0.00ed  | <0.10           |
| Onil (2)       | 8.07 ± 0.06e  | 0.53 ± 0.06e    | 0.40 ± 0.00ad | 0.30 ± 0.00ed  | 0.13 ± 0.06     |
| Picual (35)    | 3.33 ± 0.62a  | 0.61 ± 0.08ab   | 0.38 ± 0.04ac | 0.22 ± 0.04e   | <0.10           |

n: number of samples; a, b, c, d, e, f: Different superscripts for the same quality parameter mean significant differences among varieties.
Table 5  Mean and standard deviation for the sterol and triterpenic dialcohols (erythrodial + uvaol) composition (%) for the different olive varieties.

| Variety (n)                  | Cholesterol   | Campesterol | Stigmasterol | Apparent β-Sitosterol | Δ7-Stigmastenol | Total sterols (mg/kg) | Erythrodial + Uvaol |
|------------------------------|---------------|-------------|--------------|-----------------------|-----------------|-----------------------|---------------------|
| Arbequina (10)               | 0.11 ± 0.03   | 3.28 ± 0.27 | 0.70 ± 0.19  | 94.61 ± 0.37          | 0.24 ± 0.05     | 1491.8 ± 171.3       | 1.29 ± 0.33         |
| Benizal (4)                  | 0.18 ± 0.08   | 4.25 ± 0.25 | 0.78 ± 0.19  | 93.79 ± 0.31          | 0.30 ± 0.06     | 8488.4 ± 49.5        | 3.24 ± 0.36         |
| Castellana (12)              | 0.10 ± 0.00   | 2.95 ± 0.06 | 0.63 ± 0.19  | 95.63 ± 0.05          | 0.20 ± 0.00     | 10228 ± 80.6         | 2.30 ± 0.08         |
| Cornicabra (44)              | 0.14 ± 0.06   | 3.84 ± 0.20 | 0.48 ± 0.14  | 94.59 ± 1.58          | 0.23 ± 0.08     | 1468.6 ± 188.6       | 3.34 ± 0.88         |
| Cuquillo (4)                 | 0.13 ± 0.05   | 3.13 ± 0.19 | 0.74 ± 0.28  | 95.17 ± 0.32          | 0.28 ± 0.04     | 1446.4 ± 114.5       | 1.94 ± 0.56         |
| Injerta (2)                  | 0.14 ± 0.05   | 2.74 ± 0.08 | 0.67 ± 0.08  | 95.40 ± 0.22          | 0.34 ± 0.08     | 1335.9 ± 81.2        | 1.94 ± 0.14         |
| Manzanilla de Centro (5)     | 0.20 ± 0.09   | 3.24 ± 0.16 | 0.63 ± 0.17  | 94.81 ± 0.29          | 0.23 ± 0.11     | 10888.8 ± 102.9      | 1.99 ± 0.55         |
| Manzanilla de Sevilla (2)    | 0.11 ± 0.03   | 3.10 ± 0.16 | 0.42 ± 0.07  | 95.22 ± 0.20          | 0.23 ± 0.07     | 1660.1 ± 121.7       | 2.34 ± 0.55         |
| Manzanilla Local (6)         | 0.14 ± 0.05   | 2.91 ± 0.20 | 0.54 ± 0.11  | 95.26 ± 0.45          | 0.20 ± 0.08     | 1321.9 ± 65.2        | 2.04 ± 0.48         |
| Negrilla (2)                 | 0.13 ± 0.06   | 2.87 ± 0.12 | 0.60 ± 0.10  | 95.30 ± 0.36          | 0.23 ± 0.06     | 1352.3 ± 53.7        | 1.67 ± 0.45         |
| Onil (2)                     | 0.20 ± 0.00   | 3.90 ± 0.17 | 0.53 ± 0.06  | 94.13 ± 0.15          | 0.20 ± 0.00     | 1394.7 ± 108.6       | 2.23 ± 0.06         |
| Picual (35)                  | 0.19 ± 0.07   | 3.34 ± 0.13 | 0.50 ± 0.13  | 94.99 ± 0.30          | 0.31 ± 0.07     | 1105.0 ± 128.6       | 1.49 ± 0.46         |

n: number of samples; a, b, c, d, e, f. Different superscripts for the same quality parameter mean significant differences among varieties.

Manzanilla de Sevilla and Manzanilla Local), while Picual had the lowest gadoleic content (although not significantly different from Castellana and Cuquillo).

Injerta had the lowest palmitoleic content and the highest gadoleic content. Onil had the highest margaric and margaroleic content and the lowest linoleic content (although not significantly different from Arbequina, Cornicabra and Picual). The varieties Benizal, Castellana, Cuquillo Manzanilla de Centro and Manzanilla de Sevilla did not stand out in any fatty acid composition.

Sterol and triterpenic dialcohol composition is shown in Table 5. Content values were found within the ranges required by Regulation EU 1348/2013 (cholesterol ≤ 0.5%, campesterol ≤ 4.0%, stigmasterol ≤ campesterol, apparent β-sitosterol ≥ 93.0%, Δ7-stigmastenol ≤ 0.5% total sterols ≥ 1000 mg/kg, erythrodial + uvaol ≤ 4.5%), except for some samples of virgin olive oil belonging the variety Cornicabra as regards campesterol, and other samples belonging to the variety Benizal, as regards campesterol and total sterols.

There were not significant differences in the cholesterol content for the different varieties, and all the values found for this sterol were very low.

Benizal had the highest campesterol content, even higher than the variety Cornicabra, and both of them showed values above 4%, but lower than 4.5% (except for one sample of Benizal, with 4.7%). According to Regulation EU 1348/2013, when the campesterol content was between 4.0 and 4.5%, the stigmasterol and Δ7-stigmastenol contents should be lower than 1.4% and 0.3%, respectively, as in fact happens with all our samples. The variety Onil also showed high campesterol values, close to 4%. The high campesterol content found in these samples must be an intrinsic feature of the varieties, since the olives were picked and processed in highly controlled conditions.

These high campesterol values have been already reported for Cornicabra in the provinces of Toledo and Albacete, for Onil in the province of Albacete, and for Arbequina in certain warm Argentine regions, like La Rioja and Catamarca. The variety Injerta showed the lowest campesterol values, although not significantly different from Castellana, Manzanilla Local and Negrilla.

The lowest stigmasterol content was found for Manzanilla de Sevilla, although only significantly different from Arbequina, Benizal and Cuquillo, which showed the highest values.

Apparent β-sitosterol accounted for more than 93% of the total sterols. The variety Castellana stood out, although only significantly different from Benizal and Onil.

The variety Injerta showed the highest Δ7-stigmastenol values, although only significantly different from Castellana, Manzanilla Local and Onil.

Benizal was the only variety which did not arrive to the minimum 1,000 mg/kg established by Regulation EU 1348/2013 for total sterol content. This must also be an intrinsic feature of this variety, as explained above for campesterol. At the other end there were Arbequina, Cornicabra, Cuquillo and Manzanilla de Sevilla with some samples having more than 1,700 mg/kg.

The variety Cornicabra stood out in the triterpenic dialcohols (erythrodial + uvaol), although not significantly different from Benizal and Manzanilla de Sevilla.

The most useful parameters from Table 5 to discriminate among varieties were campesterol, stigmasterol, apparent β-sitosterol, total sterol and erythrodial + uvaol, but some varieties (Manzanilla de Centro, Manzanilla Local, Negrilla, Onil and Picual) did not stand out in any of these parameters.
4 Conclusions

The two more common varieties in Castilla-La Mancha (Cornicabra and Picual), gave remarkably bitter oils, with high total phenolic content and high oxidative stability, while the variety Arbequina, recently introduced in the region with great success, gave oils with low bitterness, low phenolic content and low stability. The other less common varieties had intermediate characteristics between those of these two groups. Fatty acid composition of Cornicabra and Picual characterised by high oleic and low linoleic acids content, while Arbequina showed the opposite, with low mono/polyunsaturated ratio. On the other hand, one sample of Benizal had campesterol contents above the established limits, and the total sterols content of Benizal was below the minimum level for virgin olive oil.

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