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Variety Characterization and Influence of Olive Maturity in Virgin Olive Oils from the Area Assigned to the Protected Designation of Origin "Aceite de la Alcarria" (Spain)

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Abstract: In this first contribution to the study of virgin olive oils from the area assigned to the Protected Designation of Origin (PDO) "Aceite de la Alcarria" (Spain), both monovarietal oils obtained under ideal conditions in a pilot plant, as well as blend oils made in the oil mills located in the study area, were characterized. Special interest was focused on the influence of the ripening state of the fruits. The oils from the *Castellana* variety, the main variety found in the study area, were characterized by a high content of tocopherols, medium oxidative stability and high content in palmitic, palmitoleic and linolenic acids. As the ripening process progresses, the oils lose fruitiness, bitterness, pungency, stability, and some organoleptic defects appear, to the point of lowering the category (from extra virgin to virgin) in some of the *Castellana* samples. Thus, early collection of olives of this variety is recommended.

Keywords: triglycerides; stability; oil quality; monovarietal oils

1. Introduction

Products under a Protected Designation of Origin (PDO) are defined as products elaborated completely in a particular place or region, whose quality or characteristics are fundamentally due to the particular geographical environment and the natural and human factors inherent in it [1]. The PDO "Aceite de la Alcarria" was constituted in 2008 [2], and is located in the northwest of the Autonomous Community of Castilla-La Mancha (Spain), between the provinces of Cuenca and Guadalajara. In this area, as a result of perfectly defined cultivation and processing techniques, olive oils with differentiated characteristics and an exceptional reputation are traditionally elaborated.

La Alcarria is a region located in the center of the Iberian Peninsula, with very different characteristics from other Spanish olive-growing regions. From the geological point of view, the landscape is dominated by limestone moors, very eroded, that originate poor soils. This aspect, together with a climate of very cold winters and hot and dry summers, originate the presence of endemic olive varieties that produce oils with differentiated quality. The principal variety is *Castellana* or *Verdeja*, which is endemic and strongly linked to the area, and represents about 90% of the total olive trees grown [3].

In addition, there are other, less prevalent, local varieties (*Gordera* and *Martin Galgo*), which are grown in small areas, and the *Manzanilla* variety, widespread across other Spanish olive oil regions, can also be found, but its incidence is low. Finally, the *Arbequina* variety, which is very common and popular in other PDOs in the northeast of Spain, has recently been introduced.

The PDO "Aceite de la Alcarria" is characterized by the low production of its olive trees, with an average of 5 kg per tree, and the low fat yield, only 18%, from its main variety, *Castellana* [3]. Therefore, it is important to counteract these factors through the pursuit of the differentiated quality of the oils produced in this area, which is as yet unstudied. It has been described that the importance of maintaining local varieties that give rise to differentiated sensory characteristics is part of the genetic heritage of Mediterranean olive regions [4]. Local varieties also play an important environmental role, as they are highly specialized varieties adapted to the area, preventing erosion and desertification of the soil, and contributing to maintain the balance of the ecosystems [5]. In addition, oils elaborated from these traditional cultivars produced in non-intensive systems in specific geographic origins are valorized by current market trends [6,7].

It is well known concerning the relation between genetic information of olive cultivars and the composition, nutritional value and sensory characteristics of olive oils [8,9]. Some studies emphasize the influence of olive cultivars on the overall chemical composition of olive oils [10,11], both major compounds like fatty acids [12] and minor compounds like polyphenols, which have been described as responsible for olive oil bitterness and pungency [13].

Thus, we evaluate the regulated physicochemical quality parameters (acidity, peroxide index and extinction coefficients K_{232} and K_{270}), and the sensory parameters (fruity, bitter, pungent and the Panel Test classification) of the oils elaborated from varieties grown in la Alcarria. In addition, to fully characterize these oils, we analyze the sensory descriptors of endemic varieties, the stability parameters (content in total polyphenols, tocopherols and oxidative stability at 100 °C) and the composition of fatty acids, triglycerides, sterols and triterpenic dialcohols of processed monovarietal oils, under ideal conditions (using an Oliomio extractor), from olives harvested at two different ripening stages (veraison, the onset of ripening, and ripe). To complete the study, we also characterize the oils made in the oil mills located in the study area, where the olives are not separated by variety.

2. Material and Methods

2.1. Selection of Plots and Trees within the Study Area

A total of 23 plots were selected according to the predominant variety and the presence of a sufficient number of isolated trees from nonpredominant varieties. The number of plots by variety were as follows: *Arbequina* = 2; *Castellana* = 15; *Gordera* = 2; *Manzanilla* = 2; *Martin Galgo* = 2. Within the same variety, the plots were separated throughout the study area. In each plot, six olive trees were selected and marked. The location of sampled plots and oil mills are shown in Figure 1.

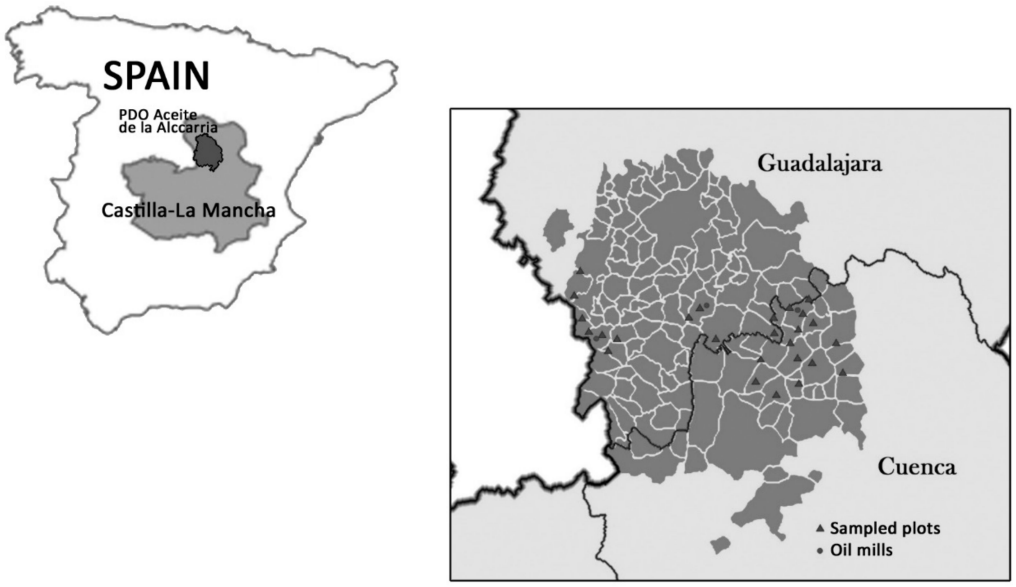

Figure 1. Location of sampled plots and oil mills within the area assigned to the protected designation of origin (PDO) Aceite de la Alcarria.

2.2. Olive Collection

Sampling was carried out at two specific moments. The first collection was conducted at the beginning of the season (first half of November), when the olives were in veraison, corresponding to a maturity index (MI) of between 0 and 2.5. The second collection was made at the end of the season (second half of December), with the MI of between 3.5 and 7 [14]. To evaluate this MI, 100 olives were selected and separated into eight categories based on their color:

0 = Skin color deep green, fruit hard
1 = Skin color yellow-green, fruit starting to soften
2 = Skin with < half the fruit surface turning red, purple or black
3 = Skin with > half the fruit surface turning red, purple or black
4 = Skin color all purple or black with all green flesh
5 = Skin color all purple or black with < half the flesh turning purple
6 = Skin color all purple or black with > half the flesh turning purple
7 = Skin color all purple or black with all the flesh purple

Then, the maturity index was calculated as the result of multiplying the number of olives of each category by the number of the color category, and dividing by 100.

$$MI = (A \times 0 + B \times 1 + C \times 2 + D \times 3 + E \times 4 + F \times 5 + G \times 6 + H \times 7)/100$$

where letters correspond to the number of olives included in each color category. In both cases, the collection began with the earliest varieties. The total number of samples was 46 (23 plots × 2 collections).

Within each plot, 20 kg of healthy olives were collected manually from the marked olive trees and immediately transported to the olive oil extraction plant to be processed.

2.3. Olive Oil Extraction

The olive oil was extracted under the best processing conditions with an Oliomio TF-30 extractor (Toscana Enológica Mori, Tavarnelle Val di Pesa, Italy). Three olive oil samples were obtained for *Arbequina*, *Gordera*, *Manzanilla* and *Martin Galgo* and 15 samples were obtained for *Castellana*.

From each sample, 2 L of oil were elaborated, filtered and decanted. The oils were stored under refrigeration in 250 mL dark glass bottles without headspace until analysis.

2.4. Sampling of Olive Oil from Oil Mills

Samples of protected designation of origin (PDO) virgin olive oil were taken directly from randomly selected storage tanks at the mills, but covering the whole harvest season. The first portion of oil was eliminated to avoid oil in direct contact with the sampler faucet. Samples were taken after the harvesting season (second half of January). A total of 16 samples were collected from the three oil mills located in the study area, depending on production capacity (four in Auñón, Guadalajara, four more in Loranca de Tajuña, Guadalajara, and eight in Valdeolivas, Cuenca).

2.5. Analytical Determinations

- Regulated physicochemical quality parameters

The regulated physicochemical quality parameters (free acidity, peroxide value and specific extinction coefficients at 232 and 270 nm–K_{270} and K_{232}) were determined according to the analytical methods described in Regulation EEC/2568/91 from the European Union Commission [15].

Acidity, expressed as g of oleic acid per 100 g of oil, was determined by titrating an oil solution dissolved in 96° ethanol-ethyl ether (1:1 *v*/*v*) with an ethanolic solution of KOH 0.1 N, using phenolphthalein as the indicator.

To measure the peroxide index, expressed as miliequivalents of active oxygen per kg of oil (meq/kg), the oil sample was mixed with acetic acid-chloroform and potassium iodide in the dark. The solution was titrated with a sodium thiosulfate solution, using starch as indicator.

The extinction coefficients, K270 and K232, were calculated by absorbance at 270 and 232 nm, with a UV spectrophotometer (Hewlett-Packard, HP 8452 A) using a 1% cyclohexane oil solution, with a quartz cuvette of 1 cm of optical path.

- Sensory analysis

The determination of sensory parameters was performed by 12 selected and trained panelists from the *Laboratorio Agroalimentario de Córdoba* (Córdoba, Spain), according to the methodology described in Regulation (EC) nº 796/2002 [16]. 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. The descriptive sensory analysis from the endemic varieties (*Castellana*, *Gordera* and *Martin Galgo*) was carried out by the taste panel from the *Instituto Tecnológico Agroalimentario* of the Extremadura Regional Government (Badajoz, Spain).

- Stability parameters

Stability parameters (total polyphenols, tocopherols and oxidative stability) were analyzed following different methodologies.

Total phenol compounds were isolated by extraction 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 extracts and the absorbances of the solution at 725 nm were measured. The concentration was determined in a calibration curve previously prepared with caffeic acid. The results were expressed in mg of caffeic acid per kg of oil [17,18].

Tocopherols were evaluated according to the American Oil Chemists' Society (AOCS) method Ce 8-89 [19]. A solution of oil in hexane was analyzed by high-performance liquid chromatography (HPLC) (HP1100) with a silica column Lichrosorb Si-60 (250 × 4.6 mm i.d. × 5 µm particle size), diluted with hexane/2-propanol (98.5:1.5), with a flow of 1 mL/min. A fluorescent detector (Waters 470) was used, with a wavelength of excitation and emission of 290 and 330 nm, respectively.

Oxidative stability was evaluated by the Rancimat method [20]. Stability was expressed as the oxidation induction time (hours), and was measured with the Rancimat 679 (Metrohm Co., Basel, Switzerland), using an oil sample of 3.5 g warmed to 100 °C and air flow of 10 L/h.

- Fatty acids and triglycerides

Fatty acid composition, expressed as the percentage (%) of methyl-esters, was determined according to Regulation EEC 2568/91 [5] and its modification EEC 1492/92 [21]. Triglycerides were hydrolyzed and transesterified to obtain the fatty acid methyl-esters. This was done by vigorously shaking a solution of oil in hexane (0.2 g in 3 mL) with 500 µL of a 0.2 N solution of KOH in methanol. Then, methyl-esters were analyzed by GC with a HP 6890 chromatograph equipped with an FID detector, using a silica column BPX (50 m × 0.25 mm i.d. × 0.255 µm film thickness). 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 the AOCS method Ch 2-91). Peaks were identified by a comparison of the retention time of standards.

Triglycerides were determined by HPLC according to Regulation EEC 2568/91 [15] and Regulation EC 2248/98 [22], and the results were expressed in equivalents carbon number (ECN). To determine triglycerides, olive oil samples were purified on a silica gel cartridge and diluted in acetone to be subsequently injected into an HPLC chromatograph (Agilent Technology1200). The detector used was an HP1040 Refractive Index (RI) detector. The sample was passed through a Teknokroma stainless steel column model TR-011439 (25 m × 0.46 mm d.i. × 0.15 µm particle size) under isocratic conditions at 40 °C, using acetone:acetonitrile 40:60 (*v*/*v*) as the mobile phase, with a flow of 1.2 mL/min. The volume of the injected sample was 10 µL.

- Sterols and triterpenic dialcohols

Sterol and triterpenic dialcohol composition, expressed as a percentage (%), was determined according to the procedure described in Regulation EEC 2568/91 [15], corresponding to protocol AOCS Ch 6-91. Samples were analyzed by GC with an HP 6890 chromatograph with a column (25 m × 0.25 mm i.d.) covered with SGL-5 (0.25 µm thickness; Sugerlabor). Working conditions were as follows: carrier gas, helium; flow 1.2 mL/min; injector temperature 280 °C; detector temperature 290 °C; oven temperature 260 °C; injection volume 1 µL. The 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 triplicate.

2.6. Statistical Analysis

The grouping and classification of the samples and the differences between varieties were determined by Principal Component Analysis (PCA), using Statgraphics Centurion XVII software.

Determinations in this study were obtained by means of three samples from varieties *Arbequina*, *Gordera*, *Manzanilla* and *Martin Galgo*, and fifteen samples from the cultivar *Castellana*. Significant differences across varieties were determined by an analysis of variance (ANOVA) which applied a Duncan test with a 95% significance level ($p < 0.05$), using SPSS software, version 11.5 for Windows.

3. Results and Discussion

3.1. Physicochemical Composition

The data from the physicochemical composition and the regulated quality parameters were used to perform the principal component analysis to evaluate differences across the virgin olive oils from the varieties analyzed (Figure 2). Clear differences were found, mainly derived from the fatty acid composition (especially palmitic, palmitoleic, stearic, oleic and linoleic), polyphenol content, tocopherols, oxidative stability and some minor compounds like campesterol and erythrodiol+uvaol. The oils from the variety *Martin Galgo* stood out from the rest, while the varieties *Arbequina*, *Gordera* and *Manzanilla*, although grouped apart, showed more similarities to *Castellana*.

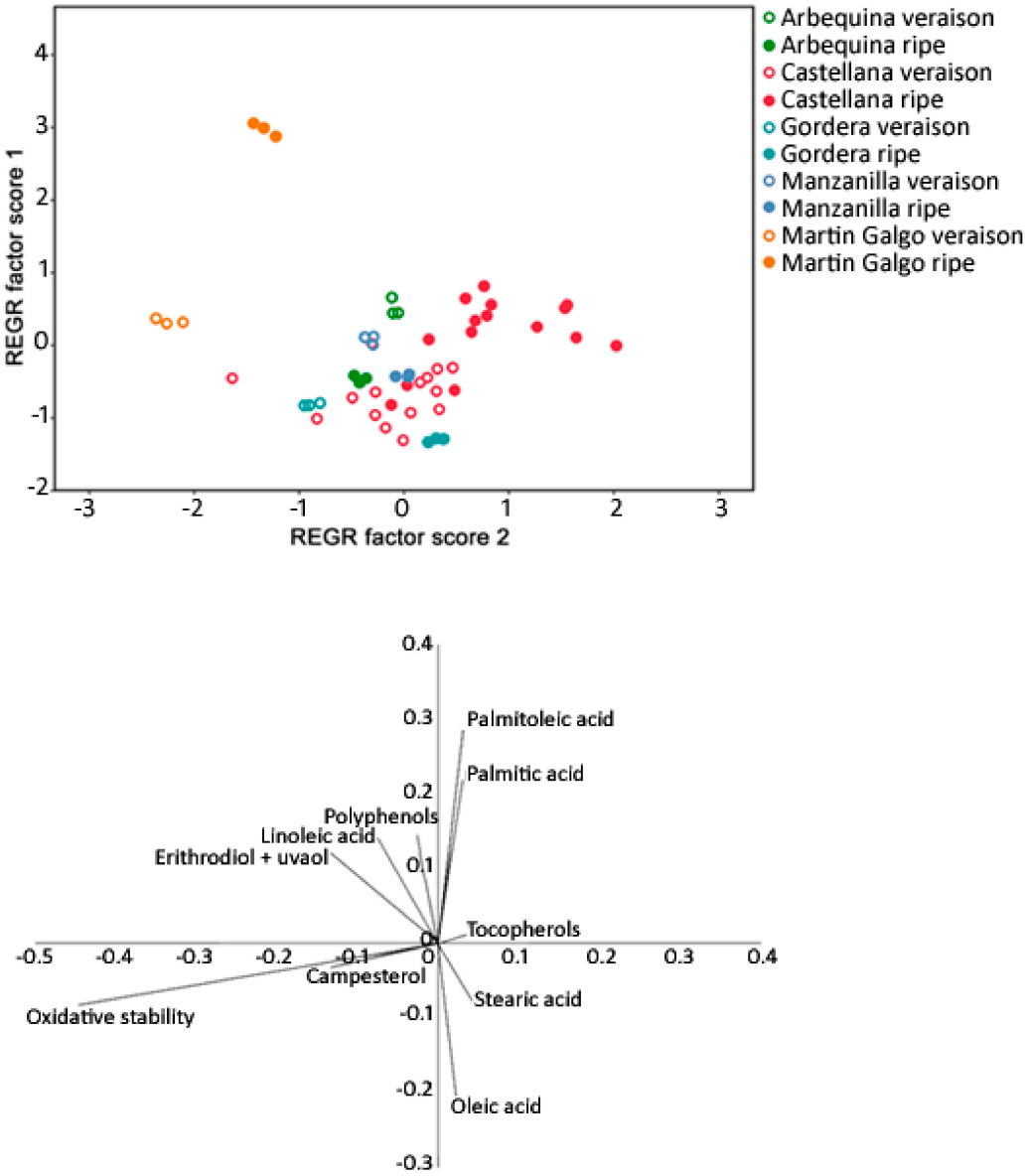

Figure 2. Principal Component Analysis (PCA) and weight of the components.

Regarding olive maturity stage, differences were also observed. In this case, in addition to the previous parameters, differences were also related to the regulated quality of the oils, with higher levels of acidity and lower stability being found for oils extracted from ripe olives.

3.2. Regulated Physical–Chemical and Sensory Parameters

Regulated physical–chemical and sensory parameters are those defined by established regulations [23]. These parameters determine possible defects in the oils or possible causes that may generate their appearance [24].

Table 1 shows the results for the regulated physical–chemical parameters from the olive oils extracted from the varieties grown in the zone, elaborated from olives in different maturity stages.

Table 1. Results obtained for the parameters of the regulated physical–chemical quality of the samples of virgin olive oil analyzed, from the different olive varieties grown in the study area, collected at different stages of maturation.

Variety **(Maturity)**	**Acidity (g/100 g)**	**Peroxide Index (meq O_2/kg)**	**K_{270}**	**K_{232}**
Arbequina (veraison)	0.10 ± 0.01 ^{b}	6.0 ± 0.60 ^{b}	0.08 ± 0.00 ^{b}	1.57 ± 0.15 ^{b}
Arbequina (ripe)	0.10 ± 0.01 ^{b}	5.0 ± 0.31 ^{b}	0.07 ± 0.01 ^{b}	1.47 ± 0.14 ^{b}
Castellana (veraison)	0.11 ± 0.01 ^{b}	5.5 ± 0.46 ^{b}	0.11 ± 0.01 ^{a,b}	1.72 ± 0.16 ^{b}
Castellana (ripe)	0.16 ± 0.01 ^{a,b}	5.3 ± 0.33 ^{b}	0.09 ± 0.01 ^{a,b}	1.54 ± 0.12 ^{b}
Gordera (veraison)	0.10 ± 0.00 ^{b}	8.0 ± 0.76 ^{a,b}	0.08 ± 0.01 ^{b}	1.68 ± 0.15 ^{b}
Gordera (ripe)	0.20 ± 0.01 ^{a}	5.0 ± 0.44 ^{b}	0.09 ± 0.00 ^{a,b}	1.43 ± 0.08 ^{b}
Manzanilla (veraison)	0.10 ± 0.00 ^{b}	4.0 ± 0.39 ^{b}	0.14 ± 0.01 ^{a}	1.75 ± 0.11 ^{b}
Manzanilla (ripe)	0.20 ± 0.01 ^{a}	10.0 ± 0.56 ^{a}	0.13 ± 0.01 ^{a}	2.13 ± 0.17 ^{a}
Martin Galgo (veraison)	0.10 ± 0.01 ^{b}	5.0 ± 0.43 ^{b}	0.06 ± 0.00 ^{b}	1.35 ± 0.13 ^{b}
Martin Galgo (ripe)	0.10 ± 0.01 ^{b}	5.0 ± 0.47 ^{b}	0.05 ± 0.00 ^{b}	1.39 ± 0.13 ^{b}

Different letters on the column indicate significant differences among cultivars. Duncan test ($p < 0.05$).

All the oils were classified as extra virgin according to regulation EC/640/2008 [25], as they showed low values on the regulated quality parameters (acidity ≤ 0.8%; peroxide index ≤ 20 meq O_2/kg; K270 ≤ 0.22; K232 ≤ 2.5).

Oils from *Manzanilla* and *Martin Galgo* showed the best values on the physical–chemical quality parameters, regardless of the maturity stage of the olives. On the contrasting values, the *Manzanilla* oils from mature olives scored highest, although in any event, these were still excellent values for an extra virgin oil.

Table 2 shows the positive sensory attributes and the Panel Test classification from the oils evaluated. Similarly to the physical–chemical quality, all the oil samples from olives in veraison were classified as extra virgin according to Regulation EC/640/2008 [25], as the median of defects was 0 and the median of the fruity attribute was higher than 0. However, the oils from mature olives showed some defects. Only 18 of the 23 samples were classified as extra virgin, while the other five were classified as virgin, as the median of defects was higher than 0 but lower than 3.5, and the median of fruity higher than 0. This finding is surprising, as the raw material was carefully collected, and the oil was elaborated within one day after collection under optimum conditions. The only variety affected was *Castellana*, which is the main variety from the area assigned to the PDO Aceite de la Alcarria. This indicates that *Castellana* olives must be collected earlier to obtain high quality oils. The main defect found was winey–vinegary, which is the characteristic flavor of certain oils reminiscent of wine or vinegar, and which is usually due to fermentation processes that take place in the olives [26]. This defect tends to appear in oils elaborated from olives in a very advanced stage of maturity. It is important to consider the significance of sensory analysis in the quality control of oils, as these samples classified as virgin presented physical–chemical parameters values from the extra virgin category.

Table 2. Results obtained for the positive sensory attributes evaluated and the Panel Test classification of the samples of virgin olive oil analyzed, from the different olive varieties grown in the study area, collected at different stages of maturation.

Variety (Maturity)	Fruity (0–10)	Bitter > 5 (% of Samples)	Pungent > 5 (% of Samples)	Classification Panel Test (% of Samples)
Arbequina (veraison)	4.5 ± 0.21 [a]	0%	0%	Extra virgin (100%)
Arbequina (ripe)	5.0 ± 0.31 [a]	0%	0%	Extra virgin (100%)
Castellana (veraison)	4.6 ± 0.17 [a]	6.7%	73.3%	Extra virgin (100%)
Castellana (ripe)	3.3 ± 0.16 [b]	0%	60%	Extra virgin (66.7%) Virgin (33.3%)
Gordera (veraison)	4.6 ± 0.14 [a]	0%	0%	Extra virgin (100%)
Gordera (ripe)	4.0 ± 0.14 [a,b]	0%	0%	Extra virgin (100%)
Manzanilla (veraison)	4.8 ± 0.27 [a]	0%	100%	Extra virgin (100%)
Manzanilla (ripe)	3.9 ± 0.25 [a,b]	0%	100%	Extra virgin (100%)
Martin Galgo (veraison)	3.4 ± 0.15 [b]	0%	0%	Extra virgin (100%)
Martin Galgo (ripe)	3.9 ± 0.15 [a,b]	0%	0%	Extra virgin (100%)
All varieties (veraison)	4.5 ± 0.20 [a]	4.3%	56.5%	Extra virgin (100%)
All varieties (ripe)	3.7 ± 0.17 [a,b]	0%	47.8%	Extra virgin (78.3%) Virgin (21.7%)

Different letters on the column indicate significant differences among cultivars. Duncan test ($p < 0.05$).

Regarding the fruity attribute, all the samples analyzed showed medium fruitiness values (fruity between 3 and 6), according to Regulation EC/640/2008 [25]. Fruity intensity from the *Castellana* oils decreased with the maturity stage, while the other varieties did not exhibit this effect. Ripening is usually associated to a decrease in C5 volatiles, which has been proposed as one of the possible causes for the decrease in green odor notes [27].

Bitter and pungent attributes were important to discriminate between the different varieties. The main variety, *Castellana*, is characterized by pungency levels over 5 (73% in veraison samples and 60% in mature samples), with only one veraison sample having a value over 5 in the bitter attribute. *Manzanilla* oils also showed high pungency levels, over 5 in all samples, while *Arbequina*, *Gordera* and *Martin Galgo* did not exceed this value for either bitterness or pungency. In general, lower values were observed in oils from mature olives (from 4.3% to 0% in samples with values over 5 on the bitter attribute and from 56.5% to 47.8% in samples with values over 5 on the pungent attribute).

3.3. Sensory Descriptors of Endemic Varieties

As regards the sensory descriptors of each of the endemic varieties grown in the study area, the results obtained were the following:

- Castellana

Medium fruity "green grass" with a slight bitterness and a medium pungency. When the olive matures, the intensity of the fruitiness of the oil decreases, evolving to ripe fruity, losing, at the same time, bitterness and pungency.

- Gordera

Medium fruity with notes of apple and fruit salad. The bitter attribute is almost imperceptible and the pungency is light.

- Martin Galgo

Light green fruity with notes of banana and almond. The bitter and pungent attributes are almost imperceptible.

3.4. Stability Parameters

Stability parameters are related to the commercial quality of the oils, which determine the maximum storage time (when bottled and distributed in commercial outlets) with good sensory characteristics [28].

Figure 3 shows the results regarding stability parameters from the virgin oils elaborated from the different varieties. All the stability parameters were useful to discriminate between varieties. The highest polyphenol content was found in oils from *Manzanilla* from olives in veraison, followed by the oil from the same variety using ripe olives with no significant differences found with oils from the variety *Castellana* from olives in veraison. On the contrasting values, oils made from ripe *Martin Galgo* olives scored lowest. When the oils were elaborated from mature olives, a significant decrease was observed regarding total polyphenols, except for *Arbequina* oils. Previous studies have reported a decrease in total polyphenol content during the maturity stage [29–31].

As regards tocopherol content, oils from the *Castellana* variety showed the highest values, regardless of the maturity stage, but with no significant differences with oils from *Manzanilla* when the oils were elaborated from olives in veraison. Tocopherols are essential compounds, as the human body cannot synthesize them, and must thus be consumed in low quantities [32]. No significant differences for tocopherol content were found related to the maturity stage of the olives.

As expected, the highest stability values were found in virgin olive oils made from *Manzanilla* olives, collected at veraison, since their content in total polyphenols and tocopherols were very high. For the same reason, they were followed in stability by oils made with ripening olives of the *Castellana* variety. When comparing the oils from veraison olives and those of mature olives, a significant decrease in the stability of the latter was observed again in the *Castellana*, *Manzanilla* and *Martin Galgo* varieties, in a similar way to the total polyphenols content. Therefore, it is proven that the oxidative stability of virgin olive oils is directly influenced by the total polyphenol content, which supports the results obtained in previous studies [30,33]. Since *Castellana* is the main variety of the study area, it would again be advisable to collect olives early in order to obtain more stable oils with a longer shelf life.

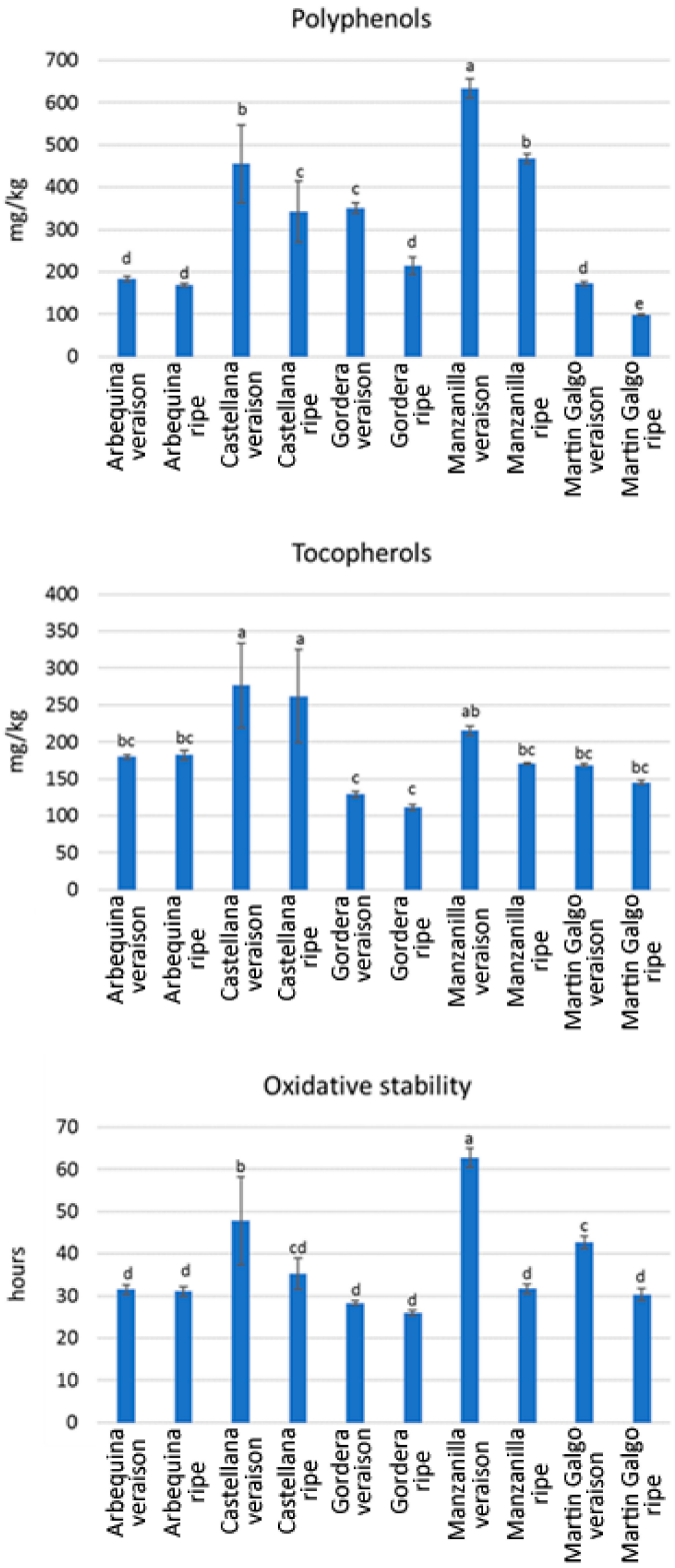

Figure 3. Stability parameters evaluated of the virgin olive oil samples analyzed, from the different olive varieties grown in the study area, collected at different stages of maturation. Different letters on the figure indicate significant differences among cultivars. Duncan test ($p < 0.05$).

3.5. Fatty Acid Composition

Fatty acid composition has a relatively wide range of values due to the genetic and environmental factors that prevail during the development of the fruit, as well as the ripeness index of the olives at the time of harvesting [34]. Fatty acid composition has been used by numerous authors as an oil classification parameter [28,33,35–40].

Table 3 shows the results obtained with respect to the composition of the main fatty acids in the virgin olive oil samples analyzed from the different olive varieties grown in the study area, collected at different times of maturation.

Table 3. Main fatty acids present in the samples of virgin olive oil analyzed, from the different olive varieties grown in the study area, collected at different stages of maturation.

Variety (Maturity)	Palmitic Acid C16:0	Palmitoleic Acid C18:0	Stearic Acid C20:0	Oleic Acid C18:1	Linoleic Acid C18:2	Linolenic Acid C18:3
Arbequina (veraison)	12.28 ± 0.76 ^{b}	1.03 ± 0.07 ^{a,b}	2.18 ± 0.20 ^{d}	75.22 ± 4.21 ^{b,c}	7.44 ± 0.39 ^{b}	0.50 ± 0.03 ^{c,d}
Arbequina (ripe)	12.00 ± 0.26 ^{b,c}	1.12 ± 0.10 ^{a,b}	1.94 ± 0.15 ^{d}	75.78 ± 3.71 ^{a,b,c}	7.53 ± 0.03 ^{b}	0.45 ± 0.01 ^{d}
Castellana (veraison)	13.64 ± 0.45 ^{a}	1.02 ± 0.03 ^{a,b}	3.07 ± 0.24 ^{b,c}	75.17 ± 1.58 ^{b,c}	5.37 ± 0.24 ^{d,e}	0.67 ± 0.06 ^{a}
Castellana (ripe)	13.91 ± 1.24 ^{a}	1.26 ± 0.02 ^{a}	3.35 ± 0.12 ^{b}	73.50 ± 6.69 ^{c}	6.47 ± 0.49 ^{c}	0.59 ± 0.01 ^{b}
Gordera (veraison)	10.95 ± 0.81 ^{d}	0.69 ± 0.01 ^{c,d}	3.22 ± 0.15 ^{b,c}	77.31 ± 6.26 ^{a,b}	5.98 ± 0.59 ^{c,d}	0.49 ± 0.03 ^{c,d}
Gordera (ripe)	12.18 ± 0.54 ^{b,c}	0.95 ± 0.04 ^{c,d}	2.83 ± 0.17 ^{c}	72.44 ± 5.80 ^{c}	9.76 ± 0.66 ^{a}	0.58 ± 0.04 ^{b}
Manzanilla (veraison)	11.74 ± 0.23 ^{b,c,d}	1.04 ± 0.09 ^{a,b}	2.11 ± 0.17 ^{d}	79.75 ± 0.91 ^{a}	3.92 ± 0.18 ^{f}	0.55 ± 0.01 ^{b,c}
Manzanilla (ripe)	11.88 ± 0.81 ^{b,c,d}	1.11 ± 0.00 ^{a,b}	2.23 ± 0.02 ^{d}	78.37 ± 2.66 ^{a}	5.04 ± 0.10 ^{e}	0.52 ± 0.03 ^{b,c,d}
Martin Galgo (veraison)	11.31 ± 0.63 ^{b,c,d}	0.64 ± 0.04 ^{d}	3.69 ± 0.13 ^{a}	77.78 ± 4.28 ^{a}	5.07 ± 0.04 ^{e}	0.59 ± 0.04 ^{a,b}
Martin Galgo (ripe)	11.23 ± 0.38 ^{c,d}	0.70 ± 0.06 ^{c,d}	3.83 ± 0.16 ^{a}	76.50 ± 4.13 ^{a}	6.25 ± 0.63 ^{c}	0.59 ± 0.03 ^{a,b}

Different letters on the figure indicate significant differences among cultivars. Duncan test ($p < 0.05$).

The values of the content of the different fatty acids in all the samples analyzed were found to be within the intervals required by Commission Regulation (EC) No. 1989/2003, regarding the characteristics of olive oils and olive pomace oils and their methods of analysis (myristic ≤ 0.05%; palmitic = 7.5–20.0%; palmitoleic = 0.3–3.5%; arachidic ≤ 0.6%; behenic ≤ 0.2%; stearic = 0.5–5.0%; oleic = 55.0–83.0%; linoleic = 3.5–21%; linolenic ≤ 1.0%) [41]. The fatty acids with the highest percentage of presence were oleic, palmitic, linoleic, stearic, palmitoleic and linolenic, which represent more than 98% of the total fatty acids in virgin olive oils sampled in the study area.

The highest palmitic acid content was found in oils made with olives of the *Castellana* variety, regardless of their ripening status, with a significant difference compared to the other oils. Laroussi-Mezghani et al. [42] reported the use of the percentages of this acid to differentiate Tunisian oils made with different varieties, as well as to differentiate these oils from others from nearby areas of the Maghreb (Algeria and Morocco).

The oils made with olives of the *Castellana* variety also stood out in palmitoleic acid content, although, in this case, significant differences were only found with the oils made from olives of the *Gordera* (veraison) and *Martin Galgo* varieties. With respect to stearic acid, the oils of the *Martin Galgo* variety showed the highest values, regardless of the ripening state of the olives, differing significantly from the rest. The lowest values for stearic acid were found in the oils made with olives of the *Manzanilla* and *Arbequina* varieties.

The oleic acid content separated the varieties studied into three groups: the first, *Manzanilla* and *Martin Galgo*, with the highest values; the second, *Arbequina* and *Castellana*, with the lowest values; and the third group, formed only by *Gordera*, whose values depended on the state of ripening of the olives, since in veraison the content of oleic acid was high, but low when the olives were ripe.

As regards linoleic acid, the oils obtained from olives of the *Gordera* variety when harvested in the mature state had the highest values, followed by those of the *Arbequina* variety, regardless of the ripening state of the olives. The lowest linoleic acid values were found in oils made with olives of the *Manzanilla* variety. Furthermore, with regard to linolenic acid, the oils obtained from olives of the

Castellana variety stood out when collected in veraison, although they did not differ significantly from the oils obtained from olives of the *Martin Galgo* variety, regardless of their state of ripening.

The low oleic acid content and the high linoleic acid content of the oils made with olives of the *Castellana* variety, the main one in the study area, together with the low total polyphenol content, when compared with the predominant varieties of the olive regions close to Alcarria (*Picual* and *Cornicabra*) [28,33], seems to be primarily responsible for their low oxidative stability (Figure 2). In fact, the oleic:linoleic ratio is frequently used as a parameter of the nutritional quality of oils and their oxidative stability [31,43].

The ripening state of the olives did not significantly affect the fatty acid content, except for linoleic acid, whose content increased as the ripening of the olive progressed. Previous studies have shown large discrepancies regarding the behavior of linoleic acid in oils from mature olives. For example, linoleic acid content also increased in the study conducted by Baccouri and colleagues [44], but decreased in that by Fuentes and colleagues [31].

The content of myristic, margaric, margaroleic, gadoleic and behenic acids was very low in all the samples of virgin olive oil analyzed, with no significant differences with respect to the variety or the state of ripening of olives.

As regards the degree of unsaturation of fatty acids, the *Castellana* variety is clearly differentiated from other varieties, regardless of the state of maturity. This difference is defined by a higher percentage of saturated fatty acids (palmitic and stearic, mainly), and a lower percentage of unsaturated fatty acids.

3.6. Triglyceride Composition

Triglycerides are the main component of virgin olive oil. The triglyceride profile has been used on numerous occasions to characterize certain olive oils [45,46] and olive varieties [47], as well as to differentiate production areas [48,49] and to detect possible adulterations with other types of vegetable fats and oils [50,51].

Figure 4 shows the results obtained with respect to the main triglycerides present in the virgin olive oil samples analyzed from the different olive varieties grown in the study area, collected at different times of maturation.

The most commonly found triglyceride was triolein (OOO), with average values ranging from 41.6% of the oils of the *Martin Galgo* variety made with olives harvested in the state of veraison, to 33.2% of *Gordera* variety oils made with olives harvested in the mature state. The *Manzanilla* and *Martin Galgo* varieties exhibited the highest values on this triglyceride. The second triglyceride in importance was POO, with values ranging from 27.2% of the *Manzanilla* variety oils, to 20.1% of the *Gordera* variety oils, both made with olives in the veraison state. The *Castellana* variety, regardless of the state of maturity of the olives, presented the highest values for this triglyceride. As for the OLO triglyceride, third in importance, the average values ranged between 19.4% of the *Gordera* variety oils made with olives harvested in the mature state, and 11.7% of the *Manzanilla* variety oils made with olives harvested in the state of veraison. *Gordera* and *Arbequina* varieties had the highest values for this triglyceride.

Within the same variety, except for *Arbequina*, the triolein of the oils decreased as the olives ripened, although without significant differences. The same applies to OOP, although, in this case, the exception was *Gordera*. These results coincide with those observed by Baccouri and colleagues [44], although these authors found significant differences. The possible explanation for this would be the decrease in the activities of lipophosphatase acyltransferase and glycerol-3-phosphate acyltransferase enzymes [52]. With respect to the OLO triglyceride, the opposite occurs, with the percentage increasing as the olives ripened. However, no significant differences were found in this case.

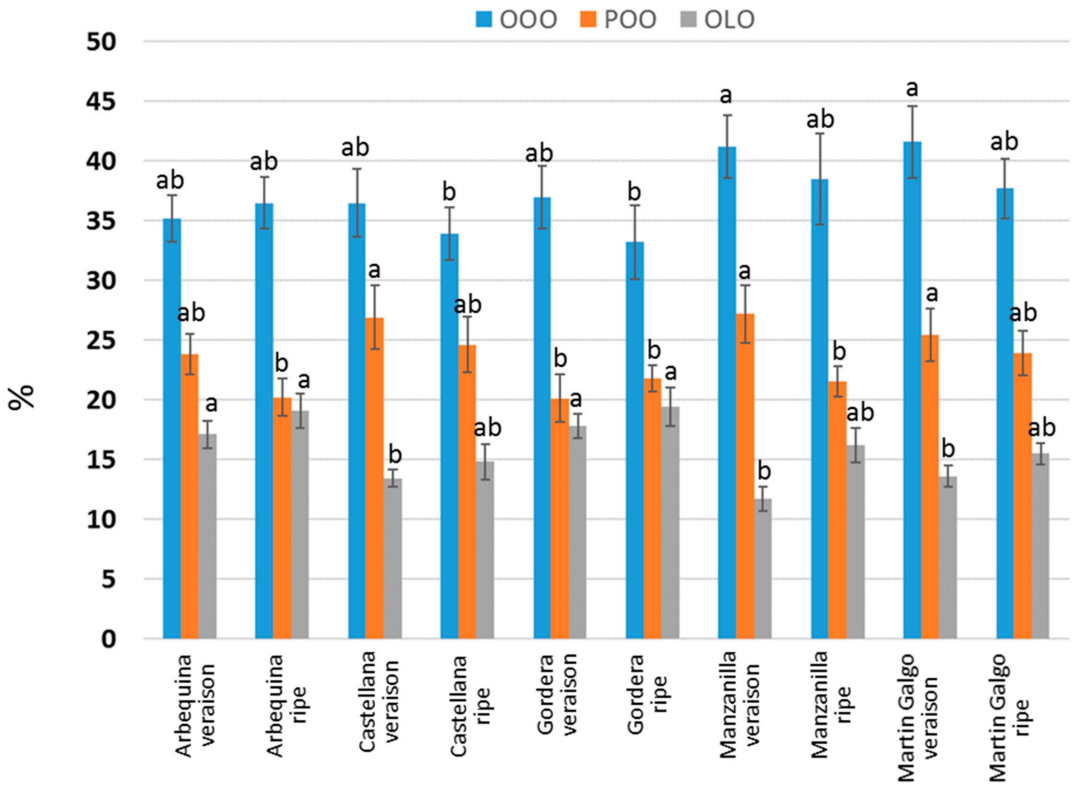

Figure 4. Main triglycerides present in the samples of virgin olive oil analyzed, from the different olive varieties grown in the study area, collected at different stages of maturation. Triglyceride composition in which L represents linoleic acid; P, palmitic acid and O, oleic acid. Different letters on the figure indicate significant differences among cultivars. Duncan test ($p < 0.05$).

These ranges of values are within those found by other authors when analyzing virgin olive oils from different sources [30,46,49,53,54]. Other triglycerides found in the different virgin olive oils analyzed, and their range of values, were as follows: PPO (2.8–4.8%), SOO (3.2–5.9%), OLL (1.5–4.8%), PPL (0.7–1.8%), SLL + PLO (5.3–8.7%), OLnO + PLL (2.3–2.9%) and POS + SLS (0.8–1.5%).

3.7. Triterpenic Sterol and Dialcohol Composition

Sterols are monovalent higher alcohols that are part of the unsaponifiable fraction of olive oil. The composition of the steric fraction of olive oil is a highly useful parameter for detecting adulterations, since it constitutes a true fingerprint of oils [55–58]. The use of these profiles has been proposed to classify virgin olive oils according to their variety [48,59–61].

Triterpenic dialcohols (erythrodiol + uvaol) are also constituents of the unsaponifiable fraction of olive oil, and are usually analyzed together with the sterol fraction. They are mainly found in the olive skin [59], so they are not extracted when pressure or centrifugation are used in the olive oil extraction process, but are extracted when organic solvents, such as hexane, are used for olive oil production. Their content is limited by Regulation EEC 2568/91 to a maximum of 4.5% of the total sterols [15]. High concentrations indicate possible adulteration with pomace oil, and therefore constitute another important index of authenticity [62].

Table 4 shows the results obtained with regard to the main triterpenic sterols and dialcohols in the composition of the analyzed samples of virgin olive oil made from the different olive varieties grown in the study area, collected at different times of maturation.

Table 4. Composition in the main sterols and triterpenic dialcohols of the samples of virgin olive oil analyzed, from the different olive varieties grown in the study area, collected at different stages of maturation.

Variety **(Maturity)**	**Campesteol (%)**	**Apparent β-Sitosterol (%)**	**Total Sterols (mg/kg)**	**Erythrodiol + Uvaol (%)**
Arbequina (veraison)	3.1 ± 0.09 ^{a}	94.9 ± 5.22	1195 ± 60.95 ^{a}	1.4 ± 0.06 ^{a,b}
Arbequina (ripe)	2.9 ± 0.04 ^{a}	94.8 ± 5.59	1096 ± 67.95 ^{a}	1.4 ± 0.03 ^{a,b}
Castellana (veraison)	2.8 ± 0.10 ^{a}	95.2 ± 5.33	1002 ± 81.16 ^{b}	2.4 ± 0.02 ^{a}
Castellana (ripe)	2.9 ± 0.11 ^{a}	95.0 ± 7.60	1009 ± 98.88 ^{b}	2.1 ± 0.06 ^{a}
Gordera (veraison)	2.5 ± 0.13 ^{a,b}	95.6 ± 8.51	1017 ± 91.53 ^{b}	2.4 ± 0.12 ^{a}
Gordera (ripe)	2.6 ± 0.05 ^{a,b}	93.5 ± 6.64	1010 ± 93.93 ^{b}	1.9 ± 0.08 ^{a}
Manzanilla (veraison)	2.2 ± 0.09 ^{b}	95.7 ± 9.28	1022 ± 85.85 ^{b}	1.4 ± 0.06 ^{a,b}
Manzanilla (ripe)	2.4 ± 0.05 ^{a,b}	95.6 ± 5.64	1020 ± 92.82 ^{b}	1.6 ± 0.03 ^{a,b}
Martin Galgo (veraison)	3.2 ± 0.15 ^{a}	94.5 ± 5.10	900 ± 72.00 ^{c}	0.8 ± 0.02 ^{b}
Martin Galgo (ripe)	3.1 ± 0.07 ^{a}	94.5 ± 5.39	900 ± 54.90 ^{c}	0.8 ± 0.01 ^{b}

Different letters on the column indicate significant differences among cultivars. Duncan test ($p < 0.05$).

The values of triterpenic sterol and dialcohol content were found to be, in all the samples analyzed, within the intervals required by Commission Regulation (EC) No. 1989/2003 regarding the characteristics of olive oils and olive pomace oils (cholesterol ≤ 0.5%; campesterol ≤ 4.0%; stigmasterol < campesterol; β-apparent sitosterol ≥ 93.0%; Δ-7-stigmastenol ≤ 0.5%; total sterols ≥ 1000 mg/kg; erythrodiol + uvaol ≤ 4.5%), if we exclude the total sterol content in the *Martin Galgo* oils.

As regards the content of campesterol, the lowest values were found in virgin oil samples made from the *Manzanilla* variety, especially when in the veraison state, while the other varieties showed significantly higher values, although still well below the maximum required by current regulations. Similar patterns have been found in Extremadura oils [62]. The apparent β-sitosterol content was very similar in all the samples analyzed, without showing, therefore, any significant differences. The total sterol content was very low in practically all the samples evaluated, very close to the minimum required (1000 mg/kg) by the current regulation (EC Regulation No. 640/2008).

The oils made from the *Martin Galgo* variety showed lower values, which does not mean it constitutes a fraud oil, as it seems to be an intrinsic characteristic of this variety. The only variety with average values for this parameter was *Arbequina*, although much lower than those found in nearby olive regions [28,38,63]. In the analysis of the triterpenic dialcohols (erythrodiol + uvaol), the *Martin Galgo* variety stood out again, with very low values, although without significant differences from the *Arbequina* and *Manzanilla* varieties.

The state of ripening of the olives did not influence the sterols and triterpenic dialcohols analyzed in any of the varieties studied, which is inconsistent with certain studies carried out on oils made with olives of different varieties. Salvador and colleagues [64], in oils made with the *Cornicabra* variety, and Fuentes and colleagues [31] in Extremadura oils, found a decrease in total sterols, and in the apparent β-sitosterol and erythrodiol + uvaol content, respectively, as olives ripen. This is probably because sterols are synthesized in the early stages of the development of the fruit, so are diluted with the subsequent fat accumulation.

The cholesterol, stigmasterol and Δ-7-stigmastenol content was very low in all the virgin olive oil samples analyzed, showing virtually no significant differences with respect to the variety or the ripening state of the olives.

3.8. Physical–Chemical Characterization of Virgin Olive Oils Collected from Oil Mills Located in the Study Area

All the virgin olive oils analyzed showed values below the regulated physical–chemical quality parameters considered (free acidity ≤ 0.8°; peroxide index ≤ 20 meq O_2/kg; K270 ≤ 0.22; K232 ≤ 2.5), so all of them were classified within the category of "extra virgin". These values confirm the physical–chemical quality of virgin olive oils in the study area.

Regarding the parameters of regulated sensory quality, 62.5% of the analyzed samples were classified in the "extra virgin" category (the median of the defects was 0 and the median of the fruity attribute greater than 0). The remaining 37.5% were listed as "virgin" (the median of the defects was greater than 0 and less than or equal to 3.5 and the median of fruitiness greater than 0). All the samples of olive oil belonging to the "virgin" category were collected from end-of-season deposits, confirming the loss of quality of the oils obtained from olives of the *Castellana* variety as the harvest period progresses. Again, these results lead to us to recommend the early harvest of olives to obtain a high-quality oil. The sensory defect found in virgin olive oil samples was the winey attribute.

Regarding sensory attributes, 50% of the samples analyzed presented high intensities (above 5) of the bitter attribute, 75% high intensities (above 5) of the pungent attribute, and another 50% (corresponding to the samples collected from the Valdeolivas oil mill-Cuenca), high intensities of both the bitter and pungent attributes. Moreover, 25% of the samples, corresponding to those collected from the Auñón mill (Guadalajara), did not present high intensities (above 5) of either the bitter or the pungent attribute.

The predominance of the *Castellana* variety in the study area was the main cause of the medium values of oxidative stability found in the oils analyzed, always higher in the samples collected in the deposits at the beginning of the campaign. This medium stability is motivated by a medium content of total polyphenols and a high content of tocopherols. The greatest oxidative stability was achieved for the oils collected in the Valdeolivas mill (Cuenca), although still far from that obtained with varieties such as *Picual* and *Cornicabra*, predominant in other nearby olive-growing areas [28,33,38]. However, the values obtained guarantee a considerable commercial value of virgin olive oils from the study area.

The fatty acid composition of the olive oils collected directly at the oil mills showed similar values to those observed in our analysis for the *Castellana* variety. The oils of the study area stood out for their high content of palmitic and palmitoleic acids, for their low content of oleic acid, and for their medium values with respect to stearic and linoleic acids. The majority triglycerides remain, in order of content, OOO, POO and OLO.

The composition in sterols and triterpenic dialcohols of the olive oils from the mills showed, similarly to the fatty acid content, values similar to those observed for the *Castellana* variety: high values of campesterol, although much lower than those found in the *Cornicabra* variety [28,33], low values with respect to total sterols and high values of erythrodiol + uvaol.

4. Conclusions

The present study covers the analysis of oils obtained from varieties grown in the area assigned to the Protected Designation of Origin (PDO) "Aceite de la Alcarria" (Spain). The aim of this study is to characterize at the same time monovarietal oils obtained under ideal conditions in a pilot plant and blend oils made in the oil mills located in the study area and compare the obtained qualities.

According to our results, special attention must be paid to the ripening state as it determines the quality of oils, especially in the *Castellana* variety. As the ripening process progresses, oils obtained from this *Castellana* variety lose fruitiness, bitterness, pungency, stability and some organoleptic defects appear, to the point of lowering the category (from extra virgin to virgin). Analysis of oils obtained in the mills show the same results confirming the loss of quality of the oils obtained from olives of the *Castellana* variety as the harvest period progresses. *Castellana* is the main cultivar of the PDO, and by optimizing its collection time, the PDO could significantly increase the quality of its production.

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