



# Olive oil characteristics of eleven cultivars produced in a high-density grove in Valladolid province (Spain)

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## Abstract

The olive oil composition of eleven cultivars (cvs. Arbequina, Arbosana, Arroniz, Cornicabra, Frantoio, Hojiblanca, Manzanilla, Picual, Picudo, Redondilla and Royuela) cultivated in a high-density olive grove located in a non-traditional producing region (Valladolid, Spain) was studied during four consecutive crop seasons. The results showed that the 122 monovarietal olive oils studied could be classified as extra virgin oils, according to the quality parameters. Furthermore, the olive cultivar had a marked effect on the chemical composition and antioxidant activity. In general, olive oils from cvs. Picual and Cornicabra showed the highest total phenolic contents, oxidative stability, and tocopherols contents, whereas the worst results for oxidative stability were observed for cv. Redondilla olive oils, probably associated to the lowest contents of phenolics and tocopherols and the highest amounts of polyunsaturated fatty acids. Finally, it was found that, despite the different crop years evaluated, the quality-chemical profiles could be used to successfully discriminate the monovarietal oils under study (linear discriminant analysis sensitivities:  $92 \pm 5\%$  for the repeated K-fold cross-validation), using the data of 15 non-redundant variables identified by the simulated annealing variable selection algorithm.

**Keywords** Monovarietal olive oils · Quality parameters · Chemical composition · Oxidative stability · Cultivar discrimination

## Introduction

Olive (*Olea europaea* L.) is one of the fastest-growing crops worldwide, mainly due to the increased popularity and consumption of olive oil worldwide, its appreciated sensory characteristics and the recognized positive effect of its consumption on humans health.

Traditionally, this crop is associated with countries of the Mediterranean basin, representing more than 90% of the world area occupied by this crop, being Spain the first world

producer [1]. However, in recent decades, the olive production system has suffered deep changes. In several areas, the traditional olive groves were progressively replaced or converted into new plantations with higher plant intensification or, in some cases, even abandoned. Thus, super-intensive hedgerows or high-density olive groves, with more than one thousand plants per hectare, have emerged, namely in hedgerow regions [2]. Furthermore, alongside improving crop yield, best management practices have been implemented, namely a more efficient use of production factors with increased irrigation, proper fertilization, and better control of pests and diseases [3, 4]. Therefore, high-density systems have been adopted due to the easiness of mechanization, high productivity, and lower production costs [5]. Recently, another trend has arisen in traditional producing countries, consisting of the expansion of olive plantation towards non-traditional regions, due to land and water availability. In Spain, this tendency is marked and has led to a successive expansion to coldest areas located in the center and north-central of the country, like the provinces of Toledo [5] and Valladolid [6]. However, it is known that climatic conditions,

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geographical characteristics, and agricultural practices, have a marked influence on the growth and productivity of the olive tree and so, on the oils' chemical composition, which may even differ between oils produced from olives of the same cultivar but grown in different geographical regions [7]. Nevertheless, to date, most of the studies regarding olive oils obtained from well-known Spanish olive cultivars have been focused on traditional Spanish cultivation regions (e.g., south of Spain) and on a restricted number of cultivars [7].

In this context, Valladolid, a Spanish region located in the north-central of the Iberian Peninsula, has gradually emerged as a new region for olive growing. Thus, a detailed study regarding olive oils extracted from a vast number of cultivars during several crop years would highlight the critical role that this region may have within the Spanish olive and olive oil sector. Therefore, this study intends to evaluate the composition of monovarietal olive oils extracted from 11 olive cultivars (*cvs.* Arbequina, Arbosana, Arroniz, Cornicabra, Frantoio, Hojiblanca, Manzanilla, Picual, Picudo, Redondilla and Royuela), grown in high-density groves in the province of Valladolid (Spain) during a consecutive 4-year crop season. It is also aimed to verify the possibility of identifying the physicochemical parameters that would possess the most cultivar-discrimination potential for correctly classifying the monovarietal olive oils, independently of the crop year effect on the oils' composition variability.

## Materials and methods

### Olive oil samples

Olive samples were obtained from an experimental high-density olive grove located in the Medina de Rioseco municipality (41° 52'48.3 "N 5° 00'17.9" W), province of Valladolid (Spain). The olive grove was installed in October 2009, in a plant density of 1666 trees ha<sup>-1</sup>, with a distance per plant of 1.5 m in the row and 4.0 between rows. Eleven different cultivars were planted, namely: *cvs.* Arbequina, Arbosana, Arroniz, Cornicabra, Frantoio, Hojiblanca, Manzanilla, Picual, Picudo, Redondilla and Royuela that were grown under the same agronomic and environmental conditions. During four consecutive crop years (2012/2013 to 2015/2016), three independent samples ( $\approx$  3 kg each) of each cultivar were hand-picked in late October / early November, when fruit ripeness varied from red spots in less than half (maturation index two) and red or purple in more than half of the olive (maturation index three) [8] and immediately transported to the laboratory of the Agrarian Technological Institute of Castilla y León (ITACyL), Valladolid (Spain). The fruits were extracted in an Abencor pilot extraction plant (Comercial Abengoa S.A., Seville, Spain) that included three main units: a mill, a thermobeaner, where the malaxation

took place at controlled temperature, and a centrifuge. The olive oils were filtered and stored in the dark in 100 mL vials at refrigeration conditions until analysis. All the assays were carried out between one and two months after extraction and were made in triplicate. In total, 122 monovarietal olive oils (Arbequina: 12 oils, Arbosana: 12 oils, Arroniz: 12 oils, Cornicabra: 9 oils, Frantoio: 12 oils, Hojiblanca: 12 oils, Manzanilla: 12 oils, Picual: 12 oils, Picudo: 12 oils, Redondilla: 9 oils, and Royuela: 9 oils), were extracted and analyzed.

### Physicochemical quality parameters

All oil samples were analyzed according to the European Union standard methods [9]. Thus, the following physicochemical parameters were assessed: free acidity (FA, in % of oleic acid), peroxide values (PV, in mEqO<sub>2</sub>/kg), as well as the specific extinction coefficients at 232 nm and 270 nm ( $K_{232}$  and  $K_{270}$ , respectively). In addition, trained panelists performed sensory analysis with no defect perceived in any of the oil samples under study. For all oils, a fruity intensity greater than 0 was detected by all panelists according to the European Union standard methods [9].

### Total phenols content

Total phenols content (TPC) was assessed according to the methodology described by Capannesi et al. [10] with the modifications described by Rodrigues et al. [11]. The determination of TPC was performed as follows: 2.5 g of olive oil were diluted with 2.5 mL of *n*-hexane (1:1 w/v) and extracted three times with methanol/water (80:20; v/v) under centrifugation (5 min at 5000 rpm). From the combined extract, 1 mL was added to 1 mL of Folin–Ciocalteu reagent and of Na<sub>2</sub>CO<sub>3</sub> (7.5%), to which 7 mL of distilled water were added. The mixtures were then stored overnight, being the spectrophotometric analysis performed at 765 nm and the concentration determined using a calibration curve established for caffeic acid in methanol (0.04–0.18 mg/mL). In accordance, the results were expressed as mg of caffeic acid equivalents per kg of olive oil (mg CAE/kg).

### Oxidative stability (Rancimat)

The oxidative stability (OS) was determined by measuring the oxidation induction time on a Rancimat 743 apparatus (Metrohm CH, Switzerland). 3.00 g of olive oil were heated at 120.0 ± 1.6 °C under an air flow rate of 20 L/h (filtered, cleaned, and dried). The resulting volatile compounds were collected in water, and the increasing of water conductivity (mS/cm) was continuously measured. The time (in hours) taken to reach the conductivity inflection curve point was recorded and corresponded to the OS value.

## Fatty acids composition

Fatty acids were assessed as their methyl esters after cold alkaline transesterification with methanolic potassium hydroxide solution [9] and extraction with n-heptane. The fatty acid profile was determined using a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, a flame ionization detector (FID), an autosampler Chrompack CP-9050 and a fused silica capillary column (CP-Sil88, 50 m × 0.25 mm i.d.; Varian, Palo Alto, CA, USA). Helium was used as carrier gas at an internal pressure of 110 kPa. The temperatures of the detector and injector were 250 and 230 °C, respectively. The split ratio was 1:50, and the injected volume was of 1 µL. The results are expressed in relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area eluting between myristic and lignoceric methyl esters. A control sample (olive oil 47,118, Supelco) and a fatty acids methyl esters standard mixture (Supelco 37 FAME Mix) were used for identification and calibration purposes (Sigma, Spain).

## Tocopherols composition

Tocopherols were evaluated following the international standard ISO 9936 [9], with some modifications as described by Rodrigues et al. [6]. Tocopherols standards ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) were purchased from Sigma (Spain), and 2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol (tocol), used as the internal standard, was from Matreya Inc. (Pleasant Gap, PA, USA). Filtered olive oil (50 mg) plus 10 µL of the internal standard solution (tocol, 100 µg/mL prepared with n-hexane) were mixed and then centrifuged for 5 min at 13,000 rpm, being the obtained supernatant was analyzed by high-performance liquid chromatography (HPLC). The liquid chromatograph consisted of a Jasco integrated system (Japan) equipped with a LC-NetII/ADC data unit, a PU-1580 Intelligent Pump, and a FP-920 fluorescence detector ( $\lambda_{\text{excitation}} = 290$  nm and  $\lambda_{\text{emission}} = 330$  nm). The chromatographic separation was achieved on a Luna Silica column (3 µm, 100 × 3.0 mm from Phenomenex, USA), operating at constant room temperature (23 °C). The eluent was a mixture of n-hexane and 1,4-dioxane (97.5:2.5) at a 0.7 mL/min flow rate. Data were analyzed with the ChromNAV Control Center–JASCO Chromatography Data Station (Japan). The compounds were identified by chromatographic comparisons with standards, by co-elution, and by the evaluation of the UV spectra. Quantification was based on the internal standard method, using the fluorescence signal response and individual calibration curves for each tocopherol. Total vitamin E was quantified as the sum of the individual tocopherol contents.

## Statistical analysis

One-way ANOVA followed by the Tukey's post-hoc multi-comparison test, when a statistically significant cultivar effect was found on the physicochemical parameters evaluated, was applied. Boxplots were also used to visualize the experimental data, being plotted the 1st, 2nd (median), and 3rd quartiles, as well as the box bars, which represented the values comprised between the 1st and 3rd quartiles. In addition, whiskers were plotted (1.5 × the inner quartile spread in length, being measured from the median), allowing establishing an arbitrary cutoff point and to identify possible outside values. Minimum and maximum values that fell outside the whisker range are also plotted (dot symbols) and symbolized possible extreme values or outliers. Linear discriminant analysis (LDA) was applied as a supervised classification technique to evaluate the possibility of the studied parameters to correctly identify the monovarietal olive oils according to the olive cultivar. The LDA was coupled with the simulated annealing (SA) algorithm to selected the non-redundant parameters with the most discriminant potential for recognizing the oils' cultivar among the oils' experimental data collected during the four consecutive crop years (i.e., FA; PV;  $K_{232}$ ;  $K_{270}$ ; TPC; OS; 12 fatty acids; and  $\alpha$ -,  $\beta$ - and  $\gamma$ -tocopherol) as well as to minimize noise effects [13, 14]. The predictive performance of the LDA-SA model was assessed based on the leave-one-out cross-validation (LOO-CV) and the repeated K-fold-CV (with 10 repeats and K set equal to 4, allowing that 25% of the data were used for validation purposes at each iteration). The percentage of correct classifications (i.e., the model's sensitivity) was calculated for both variants. 3D plot of the three main discriminant functions was used, being the class membership ellipses established using the posterior probabilities, computed using the Bayes' theorem [15]. The statistical analysis was performed using the Subselect [14] and MASS [16] packages of the open-source statistical program R (version 2.15.1), at a 5% significance level.

## Results and discussions

### Quality parameters

To establish the quality grade of the oils obtained during the four consecutive crop years (2012/2013 to 2015/2016), from the eleven cultivars under study, several parameters were determined, including the FA, the PV, and the extinction coefficient at 232 and 270 nm ( $K_{232}$  and  $K_{270}$ ). Table 1 shows, for each parameter, the mean values ( $\pm$  standard deviation) of the monovarietal oils obtained. These results, together with the fact that no defect was perceived and fruity intensity was detected by trained panelists, all oils could be classified

**Table 1** Free acidity (% oleic acid), peroxide value (mEq O<sub>2</sub>/kg), specific extinction coefficients ( $K_{232}$  and  $K_{270}$ ) of olive oils obtained from different cultivars produced in high-density olive groves (mean  $\pm$  standard deviation) in the province of Valladolid, Spain, in four consecutive crop seasons

Quality parameters	Olive cultivars											<i>P</i> -value <sup>1</sup>	
	Arbequina	Arbosana	Arroniz	Cornicabra	Frantoio	Hojiblanca	Manzanilla	Picual	Picudo	Redondilla	Royuela		
Free acidity	0.2 $\pm$ 0.06a (0.1–0.28)	0.2 $\pm$ 0.06a (0.1–0.28)	0.2 $\pm$ 0.05a (0.2–0.28)	0.2 $\pm$ 0.05a (0.2–0.28)	0.2 $\pm$ 0.08a (0.2–0.38)	0.2 $\pm$ 0.04a (0.2–0.28)	0.2 $\pm$ 0.04a (0.2–0.28)	0.2 $\pm$ 0.05a (0.2–0.28)	0.2 $\pm$ 0.05a (0.2–0.28)	0.2 $\pm$ 0.05a (0.2–0.28)	0.2 $\pm$ 0.05a (0.2–0.28)	0.2 $\pm$ 0.05a (0.2–0.28)	0.7967
Peroxide value	2.4 $\pm$ 0.97b (0.8–4.1)	3.9 $\pm$ 0.93ab (2.5–5.0)	3.9 $\pm$ 1.76ab (1.7–6.6)	3.9 $\pm$ 0.95ab (2.5–5.0)	3.1 $\pm$ 1.60b (0.8–5.0)	3.8 $\pm$ 1.49ab (2.1–5.8)	4.0 $\pm$ 0.77ab (2.5–5.0)	5.4 $\pm$ 1.78a (3.3–7.5)	4.6 $\pm$ 3.44ab (1.7–9.1)	4.3 $\pm$ 1.98ab (1.7–6.6)	3.7 $\pm$ 1.66ab (1.7–5.8)	3.7 $\pm$ 1.66ab (1.7–5.8)	0.0105
$K_{232}$	1.1 $\pm$ 0.37a (0.6–1.5)	0.9 $\pm$ 0.23a (0.6–1.3)	1.0 $\pm$ 0.23a (0.7–1.4)	1.2 $\pm$ 0.41a (0.7–1.8)	1.2 $\pm$ 0.30a (0.8–1.6)	1.1 $\pm$ 0.40a (0.7–1.9)	1.0 $\pm$ 0.21a (0.7–1.4)	1.0 $\pm$ 0.25a (0.6–1.4)	1.0 $\pm$ 0.27a (0.7–1.4)	1.2 $\pm$ 0.39a (0.6–1.5)	1.2 $\pm$ 0.36a (0.7–1.6)	1.2 $\pm$ 0.36a (0.7–1.6)	0.3709
$K_{270}$	0.1 $\pm$ 0.04c (0.0–0.2)	0.1 $\pm$ 0.04abc (0.1–0.2)	0.1 $\pm$ 0.04bc (0.1–0.2)	0.2 $\pm$ 0.04abc (0.1–0.2)	0.2 $\pm$ 0.03ab (0.1–0.2)	0.2 $\pm$ 0.04abc (0.1–0.2)	0.1 $\pm$ 0.05abc (0.1–0.2)	0.2 $\pm$ 0.03a (0.1–0.2)	0.2 $\pm$ 0.02abc (0.1–0.2)	0.1 $\pm$ 0.02abc (0.1–0.2)	0.2 $\pm$ 0.04a (0.1–0.2)	0.2 $\pm$ 0.04a (0.1–0.2)	0.0005
Sensory analysis	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	EVOO	

For each parameter, mean ( $\pm$  standard deviation) and range of values are shown. Different letters indicate significant statistical differences at a 5% significance level (one-way ANOVA followed by Tukey's multi comparison test)

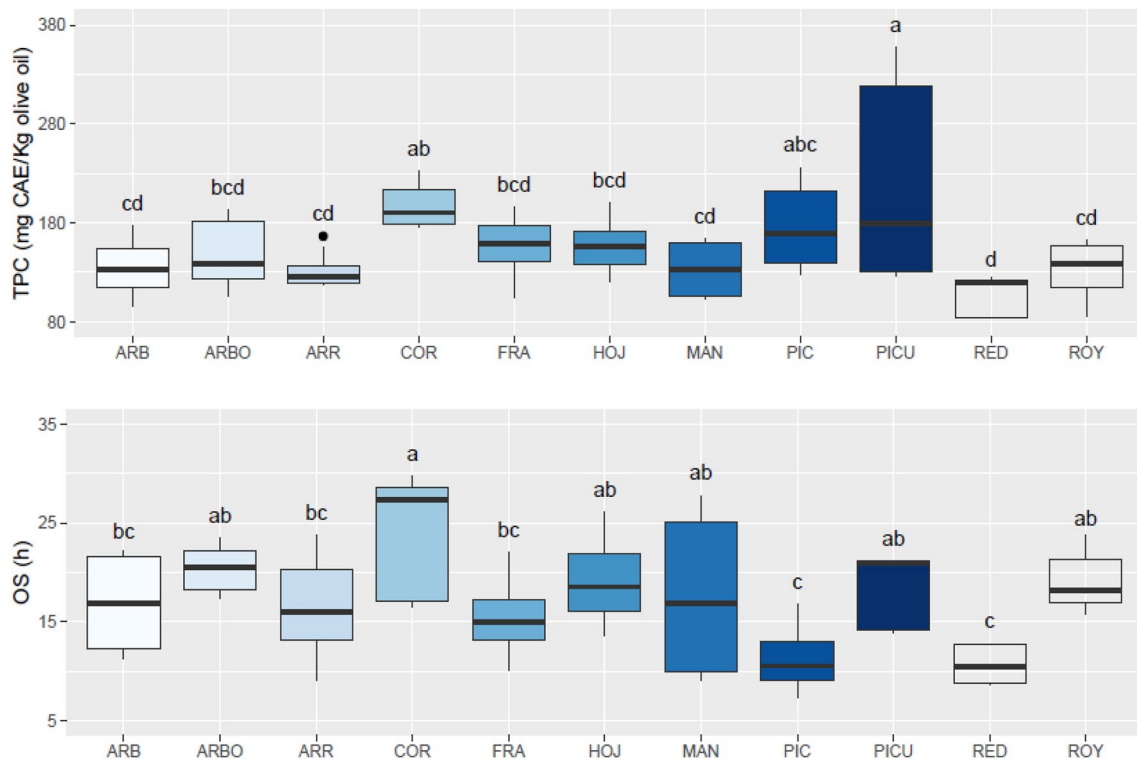
as Extra Virgin Olive Oil (EVOO) [9]. Also, it was found that the FA was not significantly different ( $P$ -value = 0.7967, one-way ANOVA) among the evaluated oils (varying from 0.2 to 0.3%). This finding is in line with the results reported in the literature for oils extracted from olives collected in high-density olive groves [6, 7, 17]. Also, no statistically significant cultivar effect was observed for the  $K_{232}$  (ranging between 0.92 and 1.24;  $P$ -value = 0.3709, one-way ANOVA). Oppositely, PV and  $K_{270}$  were significantly influenced by the olive cultivar (ranging from 2.4 to 5.4 mEq O<sub>2</sub>/kg and 0.12–0.18, respectively;  $P$ -value  $\leq$  0.0105, one-way ANOVA). Arbequina oils had the lowest PV and  $K_{270}$  mean values, and Picual oils showed the highest ones, in agreement with the values reported for these cultivars in Galicia (Spain) [17]. For the extinction coefficients the results are also in accordance with those previously reported by Mansouri et al. [18] for olive oils from three European cultivars planted in high-density groves in eastern Morocco as well as by Farinelli and Tombesi [19] for Arbequina and other four Italian olive cultivars grown in high-density hedges planted in central Italy.

### Total phenolic contents and oxidative stability

The TPC and OS of the eleven monovarietal oils produced during the four consecutive crop years are shown in Fig. 1. For TPC, a significant cultivar effect was observed ( $P$ -value < 0.0001, one-way ANOVA), being found that the cultivar that stood out with the highest TPC was cv. Picudo (mean value of 215.3 mg CAE/kg) and cv. Redondilla with the lowest levels (mean value of 109.0 mg CAE/kg). The results obtained are in line with those previously reported by Rodrigues et al. [11], for olive oils extracted from olives harvested from centennial trees located in the northeast of Portugal. Regarding the OS, which allows estimating of the oils susceptibility to oxidative degradation [20], the olive cultivar had a significant effect ( $P$ -value < 0.0001 one-way ANOVA), as can be inferred from Fig. 1. The highest value was found for cv. Cornicabra oils (mean value of 24.4 h) and the lowest one for cv. Redondilla oils (mean value of 10.6 h). It should be noticed that all OS determined were greater than the values reported by Ceci et al. [21] for Argentinian oils obtained with the same olive cultivars, highlighting the well-documented influence of the geographical origin and the edaphoclimatic conditions on the oils' chemical composition.

### Fatty acids composition

Twenty-two fatty acids (six saturated, four monounsaturated, and two polyunsaturated fatty acids, i.e., SFA, MUFA, and PUFA, respectively) were detected in the studied eleven monovarietal olive oils, extracted during



**Fig. 1** Boxplot of the total phenols (TPC) (mg CAE/kg of olive oil) and oxidative stability (OS, hours) of olive oils obtained from different cultivars produced in high-density olive groves (mean  $\pm$  standard deviation) in the province of Valladolid, Spain, in four consecutive crop seasons. Different lowercase letters indicate significant statisti-

cal differences at a 5% significance level (one-way ANOVA followed by Tukey's multicomparison test). *ARB* cv. Arbequina, *ARBO* cv. Arbosana, *ARR* cv. Arroniz, *COR* cv. Cornicabra, *FRA* cv. Frantoio, *HOJ* cv. Hojiblanca, *MAN* cv. Manzanilla, *PIC* cv. Picual, *PICU* cv. Picudo, *RED* cv. Redondilla, *ROY* cv. Royuela

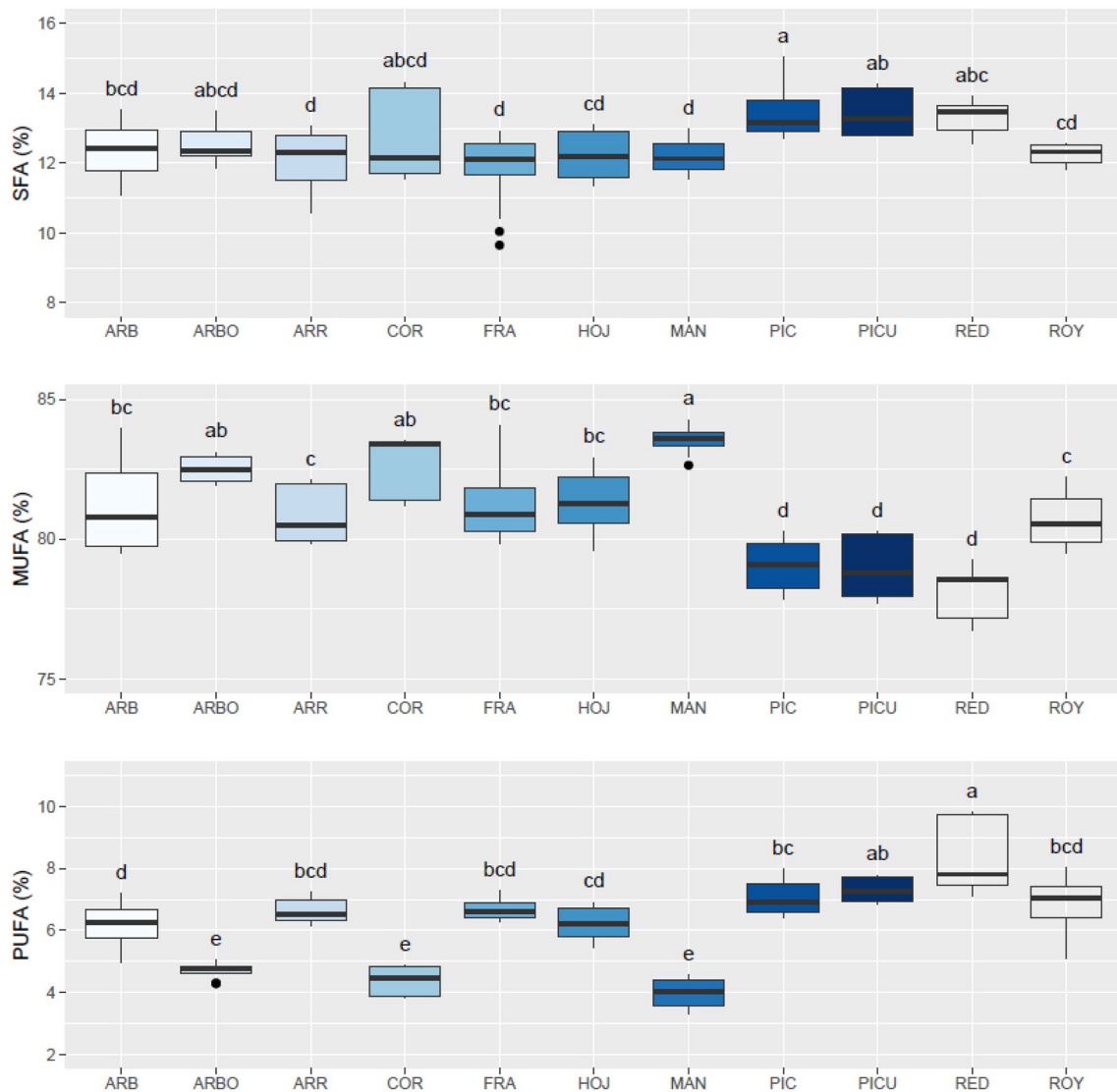
the four crop seasons, being the mean relative abundances listed in Table 2. As can be inferred, the olive cultivar had a significant statistical effect on the fatty acids relative abundance ( $P$ -value  $< 0.0001$ , one-way ANOVA), except arachidic acid ( $C_{20:0}$ ), lignoceric acid ( $C_{24:0}$ ) and gadoleic acid ( $C_{20:1}$ ) ( $P$ -value  $\geq 0.0574$ , one-way ANOVA). Globally, the most abundant was, as expected, oleic acid ( $C_{18:1}$ ), followed by palmitic acid ( $C_{16:0}$ ), linoleic acid ( $C_{18:2}$ ), stearic acid ( $C_{18:0}$ ), and linolenic acid ( $C_{18:3}$ ). According to the literature and standard regulations, these previous five fatty acids are the most abundant ones in olive oils [6, 18, 19, 22–24]. Olive oils from cv. Redondilla had the lowest oleic acid (mean abundance of 76.9%) and those from cv. Manzanilla showed the highest levels (mean abundance of 82.5%). These fatty acid relative abundances are similar to those found by Rodrigues et al. [6] but, in general, higher compared to the data reported by Farinelli and Tombesi [19] and Mansouri et al. [18] for oils produced in high-density olive groves or, more recently, by Gila et al. [24] for cv. Arbequina and Picual oils. Regarding palmitic acid, the second most abundant fatty acid, oils from cv. Hojiblanca had the lowest amounts (mean abundance of 9.1%)

and cv. Redondilla oils had the highest ones (mean abundance of 10.9%). Similar relative abundances have been described in the literature for oils obtained from different olive cultivars [6, 23, 25]. Regarding the total abundances of the SFA, MUFA, and PUFA, the values are shown in Fig. 2, being clear the significant effect of the olive cultivar ( $P$ -value  $< 0.0001$ , one-way ANOVA). The total SFA abundances varied from 11.8 (cv. Arroniz) to 13.5% (cv. Picual). The values are globally lower than those previously reported for oils produced in high-density olive groves [18, 19]. On the other hand, oils from cv. Redondilla had the lowest levels of MUFA (78.2%) and those from cv. Manzanilla showed the highest amounts (83.6%), which are, in general, greater than those reported in the literature [26, 27]. Finally, PUFA were less abundant in oils from cv. Manzanilla (4.0%) and the highest levels were found for cv. Redondilla oils (8.3%). These results are in accordance with those found by Farinelli and Tombesi [19] for Italian oils produced in high-density olive groves and by Rodrigues et al. [6] for cv. Arbequina oils obtained from when different planting densities were studied.

**Table 2** Fatty acids composition (%) of olive oils obtained from different cultivars produced in high-density olive groves in the province of Valladolid, Spain, in four consecutive crop seasons

Fatty acids (relative %)	Olive cultivars											P-value <sup>1</sup>
	Arbequina	Arbosana	Arroniz	Cornicabra	Frantoio	Hojiblanca	Manzanilla	Pical	Picudo	Redondilla	Royuela	
C <sub>16:0</sub>	9.6±0.81b (8.8–11.0)	9.6±0.58b (9.0–10.0)	9.1±0.71b (7.5–10.0)	9.1±0.93b (8.3–10.4)	9.4±1.05b (7.5–10.5)	9.1±0.58b (8.3–9.9)	10.0±0.65ab (9.2–10.9)	9.9±0.67ab (9.1–10.9)	10.8±0.87a (9.9–12.0)	10.9±0.61a (9.9–11.7)	9.2±0.39b (8.3–9.9)	<0.0001
C <sub>16:1</sub>	0.6±0.07bc (0.5–0.7)	0.8±0.06b (0.7–0.9)	0.6±0.07 cd (0.5–0.7)	0.7±0.19bc (0.5–0.9)	0.7±0.15bc (0.6–0.9)	0.5±0.03de (0.4–0.5)	0.7±0.14b (0.6–1.0)	0.4±0.08e (0.3–0.5)	0.8±0.07b (0.7–0.8)	0.9±0.10a (0.8–1.0)	0.5±0.08cde (0.4–0.6)	<0.0001
C <sub>17:0</sub>	0.1±0.03b (0.1–0.2)	0.1±0.01b (0.1–0.2)	0.1±0.02b (0.1–0.2)	0.1±0.01 cd (0.0–0.1)	0.1±0.04 cd (0.0–0.2)	0.1±0.01b (0.1–0.2)	0.0±0.01d (0.0–0.1)	0.2±0.01a (0.2–0.2)	0.1±0.05c (0.0–0.2)	0.1±0.01 cd (0.0–0.1)	0.1±0.02b (0.1–0.2)	<0.0001
C <sub>17:1</sub>	0.2±0.04c (0.2–0.3)	0.3±0.02a (0.3–0.3)	0.3±0.05ab (0.2–0.3)	0.1±0.02e (0.1–0.1)	0.1±0.02de (0.1–0.2)	0.2±0.01c (0.2–0.2)	0.1±0.01de (0.1–0.1)	0.3±0.02a (0.3–0.3)	0.2±0.05d (0.1–0.2)	0.1±0.02de (0.1–0.2)	0.2±0.02bc (0.2–0.2)	<0.0001
C <sub>18:0</sub>	1.9±0.26de (1.6–2.4)	2.0±0.09cde (1.9–2.2)	2.2±0.20bcd (2.0–2.5)	2.6±0.22a (2.4–2.9)	1.8±0.08ef (1.6–1.9)	2.4±0.25ab (1.9–2.6)	1.6±0.11f (1.4–1.7)	2.6±0.29a (2.2–3.0)	1.9±0.35ef (1.6–2.4)	1.8±0.05ef (1.7–1.9)	2.3±0.28bc (2.1–2.8)	<0.0001
C <sub>18:1</sub>	80.1±1.80bc (78.5–82.8)	81.2±0.34abc (80.7–81.6)	79.7±0.86c (78.7–80.9)	81.7±1.42ab (79.7–82.8)	80.2±1.26bc (79.1–82.8)	80.4±1.10bc (70.0–81.9)	82.5±0.72a (81.2–83.5)	78.1±0.94d (77.0–79.3)	77.8±1.05d (76.4–79.0)	76.9±0.76d (75.7–77.8)	79.7±0.99c (78.8–81.2)	<0.0001
C <sub>18:2</sub>	5.6±0.76b (4.5–6.5)	3.9±0.17c (3.7–4.1)	6.0±0.45b (5.4–6.5)	3.6±0.42c (3.1–4.1)	6.0±0.40b (5.4–6.6)	5.4±0.51b (4.7–5.9)	3.3±0.37c (2.9–3.6)	6.0±0.47b (5.6–6.8)	6.2±0.50b (5.7–6.9)	7.5±1.15a (6.3–9.0)	6.0±1.24b (4.1–7.3)	<0.0001
C <sub>18:3</sub>	0.5±0.11c (0.4–0.7)	0.6±0.12bc (0.4–0.8)	0.6±0.14bc (0.4–0.7)	0.7±0.14abc (0.5–0.8)	0.6±0.12bc (0.4–0.7)	0.7±0.24abc (0.3–1.0)	0.6±0.17bc (0.4–0.9)	0.9±0.26a (0.5–1.2)	0.8±0.33ab (0.4–1.1)	0.8±0.10abc (0.6–0.9)	0.6±0.10bc (0.5–0.7)	<0.0001
C <sub>20:0</sub>	0.5±0.14a (0.3–0.7)	0.6±0.16a (0.4–0.8)	0.5±0.19a (0.4–0.8)	0.6±0.09a (0.6–0.8)	0.5±0.21a (0.3–0.9)	0.6±0.16a (0.4–0.8)	0.5±0.28a (0.3–1.0)	0.6±0.31a (0.4–1.2)	0.7±0.36a (0.4–1.2)	0.4±0.06a (0.3–0.5)	0.5±0.28a (0.3–1.0)	0.1366
C <sub>20:1</sub>	0.3±0.16a (0.0–0.4)	0.3±0.16a (0.0–0.4)	0.2±0.13a (0.0–0.4)	0.3±0.19a (0.0–0.4)	0.3±0.14a (0.0–0.4)	0.3±0.14a (0.0–0.4)	0.3±0.17a (0.0–0.4)	0.3±0.17a (0.0–0.4)	0.3±0.20a (0.0–0.5)	0.2±0.14a (0.0–0.3)	0.2±0.14a (0.0–0.4)	0.9717
C <sub>22:0</sub>	0.15±0.03bc (0.11–0.18)	0.19±0.01a (0.17–0.21)	0.12±0.02 cd (0.10–0.16)	0.18±0.03a (0.13–0.22)	0.13±0.02 cd (0.09–0.16)	0.13±0.02 cd (0.09–0.16)	0.14±0.02bcd (0.11–0.17)	0.17±0.03ab (0.12–0.24)	0.14±0.03bcd (0.10–0.17)	0.11±0.02d (0.09–0.14)	0.11±0.02d (0.08–0.13)	<0.0001
C <sub>24:0</sub>	0.1±0.02a (0.0–0.1)	0.1±0.03a (0.0–0.1)	0.1±0.02a (0.0–0.1)	0.1±0.02a (0.0–0.1)	0.1±0.02a (0.0–0.1)	0.1±0.03a (0.0–0.1)	0.1±0.02a (0.0–0.1)	0.1±0.03a (0.0–0.1)	0.1±0.05a (0.0–0.2)	0.1±0.02a (0.0–0.1)	0.1±0.02a (0.0–0.1)	0.0574

For each fatty acid, mean (± standard deviation) and range of values are shown. Different letters indicate significant statistical differences at a 5% significance level (one-way ANOVA followed by Tukey's multi comparison test)



**Fig. 2** Boxplots of the contents (%) of SFA, MUFA, and PUFA of olive oils obtained from different cultivars produced in high-density olive groves (mean  $\pm$  standard deviation) in the province of Valladolid, Spain, in four consecutive crop seasons. Different lowercase letters mean significant statistical differences at a 5% significance level

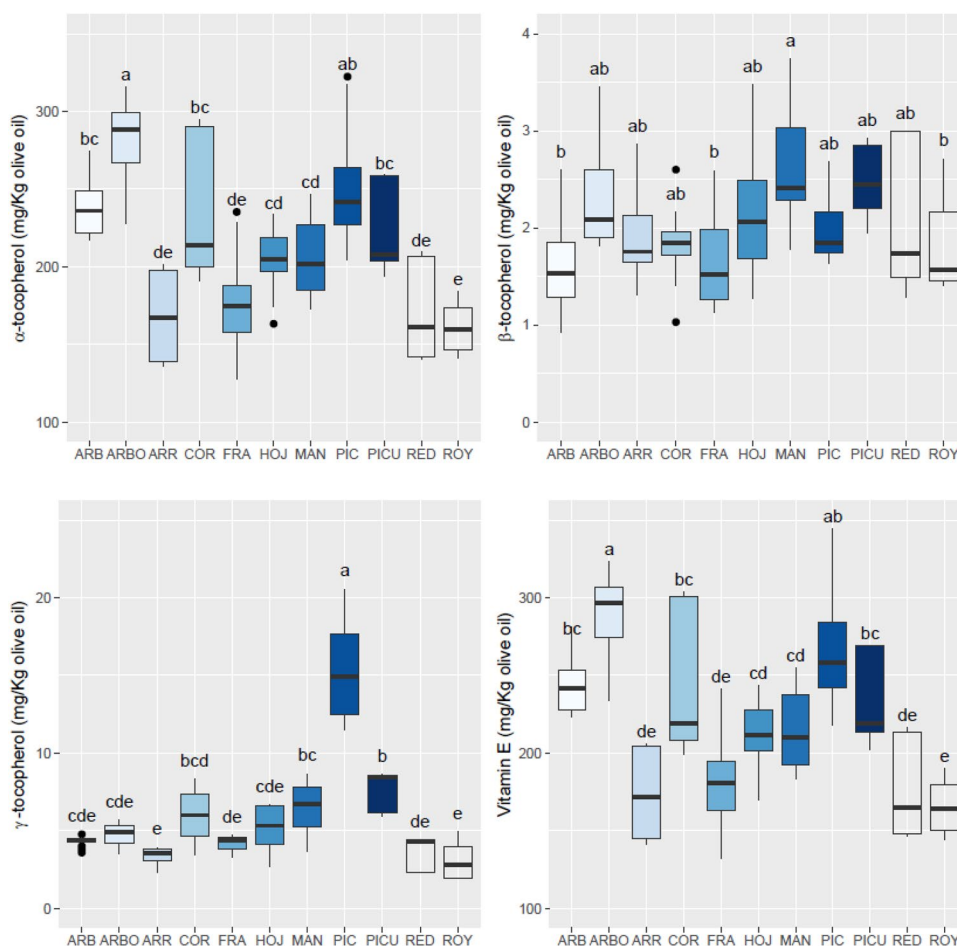
(one-way ANOVA followed by the Tukey's multicomparison test). *ARB* cv. Arbequina, *ARBO* cv. Arbosana, *ARR* cv. Arroniz, *COR* cv. Cornicabra, *FRA* cv. Frantoio, *HOJ* cv. Hojiblanca, *MAN* cv. Manzanilla, *PIC* cv. Picual, *PICU* cv. Picudo, *RED* cv. Redondilla, *ROY* cv. Royuela

### Tocopherols composition

Three tocopherols were detected in all the monovarietal oils studied ( $\alpha$ -,  $\beta$ - and  $\gamma$ -tocopherol), which contents were significantly different according to the olive cultivar, as shown in Fig. 3 ( $P$ -value  $\leq 0.0006$ , one-way ANOVA). Among them,  $\alpha$ -tocopherol was the predominant vitamin E compound (mean content from 160.9 to 279.8 mg/kg for cvs. Royuela and Arbosana, respectively). Similar  $\alpha$ -tocopherol content ranges (varying from 131.5 to 269.9 mg/kg) were reported by López-Cortés et al. [23] for traditional varietal oils (cvs. Alfafara, Farga, Morruda, and Picual) grown in eastern Spain or by Gila et al. [24] for cvs. Arbequina and

Picual oils (289.2 and 283.3 mg/kg, respectively). Regarding  $\beta$ - and  $\gamma$ -tocopherols, smaller quantities (mean contents equal or lower than 2.6 mg/kg (cv. Manzanilla) or 15.5 mg/kg (cv. Picual), respectively) were found, which are, in general, in-line with the literature data [7, 17, 24]. Since  $\alpha$ -tocopherol represented 90–95% of the total content of tocopherols, a similar trend (Fig. 2) was observed for total vitamin E amounts, which is here presented as the sum of all tocopherols. In this sense, cv. Arbosana oils had the highest mean content (286.9 mg/kg) while cvs. Redondilla and Royuela had the lowest amount (175.9 mg/kg). It is known that a higher content of vitamin E corresponds to greater resistance to oxidation [28]. Nevertheless, other factors must

**Fig. 3** Boxplots of the contents (mg/kg of olive oil) of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherols as well as of vitamin E (total tocopherol content) of olive oils obtained from different cultivars produced in high-density olive groves (mean  $\pm$  standard deviation) in the province of Valladolid, Spain, in four consecutive crop seasons. Different lowercase letters mean significant statistical differences at a 5% significance level (one-way ANOVA followed by the Tukey's multicomparison test). *ARB* cv. Arbequina, *ARBO* cv. Arbosana, *ARR* cv. Arroniz, *COR* cv. Cornicabra, *FRA* cv. Frantoio, *HOJ* cv. Hojiblanca, *MAN* cv. Manzanilla, *PIC* cv. Picual, *PICU* cv. Picudo, *RED* cv. Redondilla, *ROY* cv. Royuela



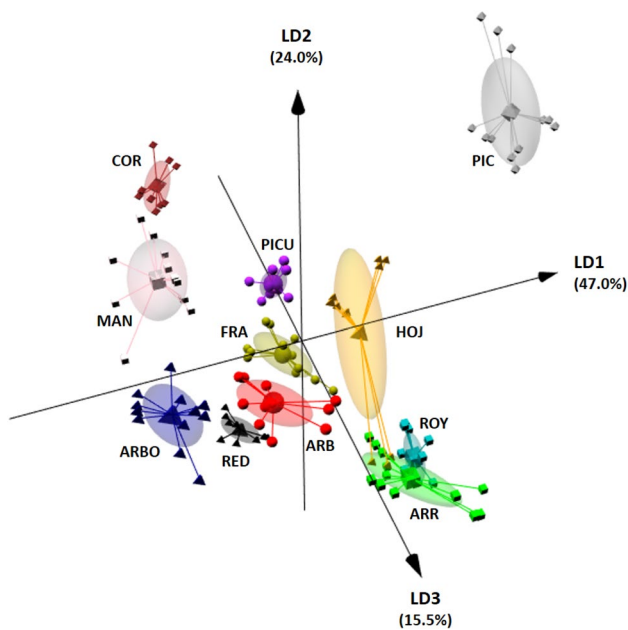
be considered to have a preponderant effect on the OS, like phenolics and fatty acid composition. The fatty acid composition was the reason that two cultivars with similar amounts of tocopherols (cvs. Redondilla and Royuela) showed significant differences in OS. Redondilla with the highest quantity of PUFA presents the lowest OS values and, in opposite, cv. Royuela possesses the lowest PUFA and high OS. Tocopherols are also important to the nutritional value of olive oil. Olive oils of cv. Arbosana together with cvs. Picual and Cornicabra, due to their high amount of tocopherols, display a high nutritional value, and their consumption would lead to better health benefits.

### Monovarietal oils discrimination based on the quality-chemical profiles

The studied monovarietal olive oils were produced during four consecutive crop years from olives collected from eleven cultivars grown in the same olive grove (same geographical region). Although the known effect of the climatic conditions varied along the four crop years, it was possible to find a significant statistical impact in most of the chemical parameters evaluated. Thus, it was further assessed the

possibility of using the established quality-chemical profiles to discriminate the monovarietal oils under study, using an LDA. Furthermore, to identify the non-redundant parameters with the most olive cultivar discriminant power, among 21 of the 25 parameters evaluated (being not included vitamin E, SFA, MUFA and PUFA, in order to minimize the risk of selecting correlated variables), the SA algorithm was implemented. The multivariate classification approach allowed establishing a LDA-SA model based on 15 parameters (FA, PV,  $K_{270}$ , TPC, OS,  $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol,  $C_{16:1}$ ,  $C_{17:1}$ ,  $C_{18:2}$ ,  $C_{18:3}$ ,  $C_{20:1}$ ,  $C_{22:0}$ , and  $C_{24:0}$ ), which three first discriminant functions explained 86.5% of the data variability. It should be noticed that among the 15 selected parameters not all were significant (e.g., FA,  $C_{20:1}$  and  $C_{24:0}$ ; as shown in Tables 1, 2). The inclusion of these variables on the classification model can be tentatively explained since, as discussed by Lo et al. [29], variables with strong prediction power sometimes fail to be significant since the statistical techniques used in each case depend on different properties of the underlying distributions. The established LDA-SA model correctly identified the oils' olive cultivar of 96.7% of the original grouped samples (one cv. Arroniz oil and one cv. Hojiblanca oil misclassified as





**Fig. 4** 3D-plot regarding the discrimination of monovarietal olive oils obtained from different cultivars (*ARB* cv. Arbequina, *ARBO* cv. Arbosana, *ARR* cv. Arroniz, *COR* cv. Cornicabra, *FRA* cv. Frantoio, *HOJ* cv. Hojiblanca, *MAN* cv. Manzanilla, *PIC* cv. Picual, *PICU* cv. Picudo, *RED* cv. Redondilla, *ROY* cv. Royuela) produced in high density-olive groves in the province of Valladolid, Spain, in four consecutive crop seasons., using a LDA-SA model established using 15 non-redundant selected parameters (FA, PV,  $K_{270}$ , TPC, OS,  $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol,  $C_{16:1}$ ,  $C_{17:1}$ ,  $C_{18:2}$ ,  $C_{18:3}$ ,  $C_{20:1}$ ,  $C_{22:0}$  and  $C_{24:0}$ ) from a dataset comprising 21 independent chemical variables

cv. Royuela oil, Fig. 4) and 93.5% of the samples for the LOO-CV procedure (four cv. Arroniz oils and one cv. Hojiblanca oil misclassified as cv. Royuela oil; one cv. Royuela oil misclassified as cv. Hojiblanca oil). Therefore, it could be inferred that the studied olive oils possessed a cultivar-dependent chemical composition that allowed the oils discrimination independently of the possible crop-year effect. The developed classification model was only less powerful for cv. Arroniz oils' identification. This finding is of utmost relevance when a cultivar genuineness assessment is envisaged for high-value monovarietal EVOO commercial classification.

The satisfactory predicted performance was further checked using the repeated K-fold-CV procedure, which enabled to retain 25% of the dataset for validation purposes. The repeated K-fold-CV (10 repeats  $\times$  4 folds) showed a mean sensitivity (i.e., correct classification percentage) of  $92 \pm 5\%$  (varying from 81 to 100%). The satisfactory performance of the proposed classification multivariate approach is in line with the results previously reported in the literature, although, in general, for a lower number of cultivars and crop years. For example, Olmo-García et al. [30] also achieved a successful olive oil discrimination according to

the cultivar using a partial least square discriminant analysis (PLS-DA) to differentiate oils produced in the USA in a single crop year, from eight different cultivars, based on phenolic and triterpenic compounds, tocopherols, sterols, and free fatty acids. Maléchaux et al. [31] described similar satisfactory classification performances for discriminating monovarietal olive oils produced from six French cultivars grown over three harvest years (2016, 2017, and 2018), using PLS-DA based on near-infrared and mid-infrared spectroscopic data used alone or combined. More recently, Özdikicierler [32] also reported the successful discrimination of oils obtained from eight olive cultivars from four different regions of Turkey using PLS-DA based on fatty acid and sterol composition.

## Conclusions

The carried out study focused on monovarietal olive oils produced in a high-density grove located in the Spanish region of Valladolid, a non-traditional olive oil producing region, characterized by its slight continental climate, with moderately cold winters and hot, sunny summers, but with cool nights, with not abundant precipitation. The results pointed out that the oils produced from the 11 studied cultivars (cvs. Arbequina, Arbosana, Arroniz, Cornicabra, Frantoio, Hojiblanca, Manzanilla, Picual, Picudo, Redondilla, and Royuela) during four consecutive crop years could be classified as extra virgin olive oils and so, possessed a similar quality grade as those usually produced in more traditional Mediterranean regions. As for the traditional cultivation geographical regions, the olive cultivar had a marked effect on the chemical composition of olive oils, mainly in monounsaturated fatty acids. Indeed, the oils' fatty acids composition stands out from oils also produced from the same cultivars but originated from more traditional olive tree cultivation areas of Spain and the world. This study also found that, despite the different cultivation years evaluated, the chemical quality profiles could be used to successfully discriminate the monovarietal oils under study, being possible to use this chemical information as putative cultivar genuineness biomarkers. Finally, the study highlighted the possibility of obtaining olive oils with superior quality in a cold non-traditional geographical region and strengthening the Valladolid region as a potential and emerging geographical area for olive oil production.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Compliance with ethics requirements** This study does not contain any studies with human participants or animals performed by any of the authors.

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