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ORIGINAL ARTICLE

Oil Content and Fatty Acid Profile of Spanish Cultivars During Olive Fruit Ripening

S. Gómez-González · J. Ruiz-Jiménez · M. D. Luque de Castro

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Abstract The oils produced from five olive cultivars (i.e. arbequina, gordal, manzanilla, picual and picudo) and the evolution of fatty acid profile during olive fruit ripening were studied. The total oil content increased throughout ripening for Picudo cultivars; however, all other cultivars reached maximum oil contents between ripeness stage 2 and 4. The content of *trans* (0.3–5.8%), saturated (8.3–21.4%), monounsaturated (41.9–84.1%) and polyunsaturated fatty acids (2.3–49.7%) varied with the ripeness state and cultivar. Different trends of the different parameters were found as a function of the ripeness state and the cultivar. Statistical analysis reveal that manzanilla drupes, at the different ripeness states, are clearly different from the rest of the cultivars. In addition, there were no significant differences in the fatty acid profile of the oils obtained from drupes at ripeness states between 2 and 4.

Keywords Fatty acids · Olive ripening · Olive oil · GC–MS · Pattern recognition

Introduction

From a botanical standpoint, olive fruit is classified as a drupe, similar to other drupes of stoned fruits such as peach or cherry. Its component parts are the exocarp or skin, the mesocarp or flesh, and the endocarp or pit, which consists of a woody shell enclosing one or, rarely, two seeds [1]. Olive oil is characterized by excellent nutritional, sensory and functional properties [2], and is considered as an agricultural product with major economical importance in the Mediterranean area [3].

Olive-drupe composition differs from sample to sample but, in general, these drupes have as major constituents water and fatty acids, and, as minor components, sugars, proteins, pectins, tannins, alcohols, sterols and flavonoids. The concentration of the different compounds is influenced to a large extent by the cultivar, soil, climate, irrigation, degree of ripeness and processing methods [4]. Some of the minor components are transferred into the olive oil during extraction; others remain with olive-oil byproducts and are discarded with them.

Depending on the saponification capability, oil components can be divided into two fractions: a saponifiable fraction, constituted mainly by triacylglycerides; and an unsaponifiable fraction, which contains minor components such as phenols, esters, terpenes, pigments and tocopherols. Olive oil produced from olives collected at optimum harvest time contains 98–99% of the first fraction and a percentage lower than, or equal to, 2% of the second fraction. Despite the small contribution of this fraction to the composition of olive oil, it has a key importance from the point of view of the biological value of the given oil.

The saponifiable fraction is composed mainly of oleic acid, a monounsaturated fatty acid, which accounts for 80% of the total lipid composition. Other major fatty acids present in olive oil are the polyunsaturated linoleic acid (2.5–20%) and the saturated palmitic acid (10–20%) [5]. This fatty acid composition of olive oil has gained much attention thanks to its beneficial implications for human

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S. Gómez-González · J. Ruiz-Jiménez · M. D. Luque de Castro (✉)
Department of Analytical Chemistry, University of Córdoba, Campus of Rabanales, Marie Curie Building, Annex C-3, Córdoba, Spain
e-mail: qa1lucam@uco.es

health. Epidemiological studies suggest that a higher proportion of monounsaturated or omega-3 fatty acids in the diet is linked to a reduction in the risk of cardiovascular diseases [6].

The individual isolation and quantification of fatty acids present in olive oil has been used for characterization and quality evaluation of the oil [7]. Greek and Italian oils have been characterized according to their fatty acid profiles [8, 9]. The fatty acid profile has also been used for identification of the geographical origin of olive oil. Extra-virgin olive-oils produced in different regions of Spain, Italy and Portugal have been differentiated through their fatty acid content [10].

The olive ripening stage is one of the most important factors associated with the quality evaluation of olive oil [11, 12]. In general, during ripening, several metabolic processes take place in olive drupes with subsequent variations of the chemical structure and concentration of some compounds. These changes are reflected in the quality grade, sensory characteristics, oxidative stability and nutritional value of the obtained product. Chlorophyll and carotenoid pigments are examples of compounds involved in this phenomenon, as well as polyphenol and fatty acid compositions. Beltrán et al. [13] suggested that the majority of olive oil produced does not belong to the best commercial quality as the fruit has not been picked at the optimal harvest time. This illustrates the need to determine an appropriate maturation stage of each olive cultivar for picking and processing.

The objectives of this study were to determine the evolution of oil content and fatty acid classes during ripening of olive fruits. In addition, statistical studies based on non-supervised pattern recognition techniques were used to differentiate olive drupes from different cultivars. In this research, the fatty acid composition of the oils produced from five olive cultivars and the evolution of these components during olive fruit ripening has been studied. Gas chromatography–mass spectrometry (GC–MS) was used for determination of the fatty acid profile. Statistical studies based on non-supervised pattern recognition techniques were developed to differentiate olive drupes from different cultivars and to identify the ripening state.

Materials and Methods

Reagents

All reagents were analytical grade or higher. Methanol and n-hexane were provided by Panreac (Barcelona, Spain), anhydrous sodium sulfate from Sigma-Aldrich (Steinheim, Germany) was used as drying agent for the non-polar phase prior to derivatization. Methanolic solutions of 0.4 M KOH and 5% H_2SO_4 were used for derivatisation of esterified and non-esterified fatty acids.

Methyl esters of acids dodecanoic (12:0), hexadecanoic (16:0), *cis*-9-hexadecenoic n7 (16:1 n7), heptadecanoic (17:0), 10 heptadecenoic (17:1 n10), octadecanoic (18:0), *trans*-9 octadecenoic (18:1 n9*t*), *cis*-9 octadecenoic (18:1 n9), *cis*-7-octadecenoic n7 (18:1 n7), *trans*, *trans*-octadecadienoic (18:2 *t*9, *t*12), *cis*, *trans*-octadecadienoic (18:2 *c*,*t*), *trans*, *cis*-octadecadienoic (18:2 *t*,*c*), *cis*, *cis*-octadecadienoic (18:2 *c*,*c*), *trans*, *trans, trans*-octadecatrienoic (18:3 *t*,*t*,*t*), *trans, trans, cis*-octadecatrienoic (18:3 *t*,*t*,*c*), *trans, cis, trans*-octadecatrienoic (18:3 *t*,*c*,*t*) *cis*, *trans*, *trans*-octadecatrienoic (18:3 *c*,*t*,*t*), *cis, cis, cis*-octadecatrienoic (18:3 *c*,*c*,*c*), *cis*, *trans–cis* octadecatrienoic (18:3 *c*,*t*,*c*), *trans*, *cis*, *cis*-octadecatrienoic (18:3 *t*,*c*,*c*), *cis*,*cis*, *cis*-octadecatrienoic (18:3 *c*,*c*,*c*), eicosatrienoic (20:3 n6), eicosatetraenoic n3 (20:4 n3), eicosapentaenoic n3 (20:5 n3) and hexacosanoic (26:0) from Sigma-Aldrich were used as standards for calibration. Nonadecanoic acid methyl ester (19:0) from Fluka (Steinheim, Germany) was used as internal standard in the determination step.

All fatty-acid analytical standards employed to prepare multistandards were supplied by Sigma-Aldrich and purchased as fatty-acid methyl esters (FAMEs) with the exception of palmitoleic, *cis*-10-nonadecenoic, hexacosanoic, octacosanoic, all *cis*-4,7,10,13,16-docosapentanoic and all *cis*-4,7,10,13,16,19-docosahexanoic, which were purchased as such acids, then methylated by using a H_2SO_4–CH_3OH solution, as described below in the section on methods for the derivatization step.

Individual stock standard solutions were prepared by diluting each compound in the appropriate solvent. Thus, high-purity compounds ranging from C9:0 to all C20 compounds were diluted in n-hexane, whereas those ranging from C22:0 to C28:0 were diluted in heptane. Stock standard solutions were stored at −20 °C.

Samples

The olive fruits used in this research were collected in the region of the Guadalquivir valley, Encinarejo (Córdoba). Five genetic cultivars of olive fruit (i.e. arbequina, gordal, manzanilla, picual and picudo) were used. The olive drupes were harvested from September 2008 to January 2009 in each of the five maturity states of the fruit, as a function of the external color of the drupe (1, green; 2, green-yellow; 3, yellow-purple; 4, purple; and 5, black) [14]. All samples were taken from the same geographical area to avoid variations caused by soil characteristics and environmental factors, taking into account that the hydrological year was dry, the hydric stress was controlled by irrigation. For representativeness, samples of approximately 250 g were handpicked from all sides of four different trees per each

cultivar, the final sample weigh was rounded to 1 kg. The drupes were milled, homogeneized by agitation in a vortex, and kept at −20 °C until use.

Extraction Methods

Fifteen grams of the milled drupes was subject to traditional Soxhlet extraction with 80-mL *n*-hexane for 24 h at 69 °C (boiling point of hexane). After extraction, hexane was removed under vacuum using a Büchi R-200-rotary evaporator and a gentle N_2 stream was used for the removal of the water traces in the oil. The oil was then weighed and subjected to the derivatization step.

Derivatization Step

The fatty acids in the oil were derivatized using the method proposed by the International Olive Council (IOC) for preparation of FAMEs [15], to convert them into more volatile compounds able to be separated by gas chromatography. The whole derivatisation method involved two steps: (1) transesterification into methyl esters with subsequent extraction of the esterified fatty acids (EFAs), and, (2) derivatization and isolation of the non-esterified fatty acids fraction (NEFAs).

The analytical sample (0.05 g oil) was diluted with 2 mL *n*-hexane, mixed with 500 μL 0.4 M KOH in methanol and homogenized for 5 min in a vortex. The mixture was left for 5 min and the hexane phase, containing most of the derivatized analytes, was transferred to a test tube. A second extraction of the oily phase was performed with 2 mL *n*-hexane, agitation in a vortex for 2 min, followed by phase separation. The two *n*-hexane extracts were mixed and diluted (1:250) with *n*-hexane containing 5 μg mL^{-1} of *c*10 nonadecenoic acid, an internal standard; 10 μl of the mixture was injected into the gas chromatograph for analysis of EFAs.

For derivatization of NEFAs into their FAMEs, a small amount of anhydrous sodium sulfate was added to the remaining fraction from the previous step to remove residual water; then, 500 μL H_2SO_4 was added and the tube was placed into a water bath thermostated at 70 °C for 30 min. After cooling, 1 mL *n*-hexane was added, agitated for 2 min in a vortex and, after phase separation, the top *n*-hexane phase containing the derivatized NEFAs was transferred to a test tube. Liquid–liquid extraction was repeated to ensure total recovery of NEFAs and the organic phase containing them was led to dryness under a stream of nitrogen, and the residue dissolved in 500 μL *n*-hexane and shaken for 2 min. Finally, after 1:75 dilution with *n*-hexane containing the internal standard, 10 μL of this solution was injected into the gas chromatograph for the analysis of NEFAs.

Separation and Identification–Determination

The appropriate separation of FAMEs by GC and identification by MS were carried out using the GC–MS method developed by Sánchez-Ávila et al. [16]. Briefly, high-purity helium (99.9%) at 1.0 mL min^{-1} was used as the carrier gas in the chromatographic step. A volume of 10 μL was injected by using a Varian SPI/1079 programmable-temperature injector (Varian, Walnut Creek, CA). The temperature program of the injector was as follows: start at 70 °C, held for 0.5 min, increase at 100 °C min^{-1} to 250 °C, and then kept for 78 min. The injection was in the split–splitless mode. The splitter was open (100:1) for 0.5 min, closed for 3.5 min and then open at 100:1 split ratio for 10 min.

The samples were analysed in an A CP-3800 gas chromatograph from Varian, furnished with a Varian CP 8400 autosampler and an SP™-2380 fused silica capillary column (60 m × 0.25 mm I.D., 0.2 μm film thickness) from Supelco (Bellefonte, PA). The oven temperature program was as follows: initial temperature 70 °C (held for 1.2 min), increased at 25 °C min^{-1} to 120 °C, followed by a second gradient of 2 °C min^{-1} to 243 °C and, finally, increased by 40 °C min^{-1} to 270 °C and held at this temperature for 5 min.

Detection of the analytes was done by a Saturn 2200 ion-trap mass-spectrometer (Sunnyvalley, TX) operating in the EI mode. Full scan and μ_Selected Ion Storage (μ_SIS, similar to Selected Ion Monitoring) were used in the experiments. The manifold, trap and transfer line temperatures were set at 60, 170 and 200 °C, respectively. The analyses were performed with a filament-multiplier delay of 11 min. Full scan acquisition, used for the identification of the FAMEs, was performed in the range *m*/*z* 40–650, with a background mass of *m*/*z* 45. Quantitation was developed using the μ_SIS mode.

The limit of detection (LOD) for each analyte was expressed as the mass which gives a signal that is 3σ above the mean blank signal (where σ is the standard deviation of the blank signal). The LODs obtained ranged between 0.2 and 20 μg kg^{-1}. The limits of quantitation, expressed as the mass of analyte that gives a signal 10σ above the mean blank signal, ranged from 0.66 to 66 μg kg^{-1}. The repeatability, expressed as relative standard deviation (RSD) calculated for five replicates, ranged between 1.80 and 6.21% for all target analytes.

Chemometric Analysis

Models based on principal component analysis (PCA) were developed to study the influence of the olive-tree cultivar and harvest period on the concentration of fatty acids. The results were confirmed by means of models based on

hierarchical cluster analysis (HCA) [17]. The concentrations obtained by the proposed method for these compounds, expressed as μg/g, were used as variables for development of the models. Statistical analysis was performed using both the raw data and those obtained after normalisation and differentiation processes. Mean normalisation X(i,k) = X(I,k)/Abs(Mean(X(i,*) and Norris derivative [segment size for averaging equal to 3; and, difference (Xk−Xk−1) equal to 2] were used to obtain normalized and derived data.

The Unscrambler 9.0 from CAMO (Oslo, Norway) was used as statistical software [14] in the case of the PCA models. Statgraphics Centurion XV, Statpoint Technologies (Warrenton, VA) was used as statistical software for the development of the HCA models.

Results and Discussion

Total Oil Content

Figure 1 shows the oil content, expressed as percentage, from the whole fruit of each cultivar as a function of the ripeness state. The maximum values obtained for the total oil content in the drupes range between 12.3 and 22.4% for manzanilla and picudo cultivars, respectively. These values are in agreement with the results obtained in a previous study [18]. As can be seen in Fig. 1, two trends were observed. The first trend was found for the picudo cultivar, where the percentage of oil in the drupes increases during ripeness and the highest value was obtained for the ripeness state number 5. The other trend involved the drupes of the rest of the cultivars. In this case, the maximum percentage of oil was obtained in ripeness states ranging between 2 and 4, as a function of the cultivar (see Fig. 1). After this optimal ripeness state, the percentage of oil obtained gradually diminishes. These behaviours were reported by Nergiz and Ergönül [19] for table olives, but similar results were also found for olive cultivars used for oil production [20].

These trends were described previously by Sánchez [21]. The profile obtained for the oil accumulation process is a function of the part of the drupe under study. While the oil accumulation in the endocarp is slow and can reach a plateau during the optimum ripening state, the oil accumulation in the seed is a fast process and, once the maximum oil content is achieved, a slight decrease may take place.

Fatty Acid Profile

The complexity and quantity of data obtained from the fatty-acid profile made it impossible to use the raw data for characterization and comparison of the oils obtained from the different ripeness states. To overcome this problem, fatty acid classes such as *trans*, saturated, monounsaturated and polyunsaturated fatty acids were used (Table 1). The quality of the oils obtained from the different ripeness states was based on these classes.

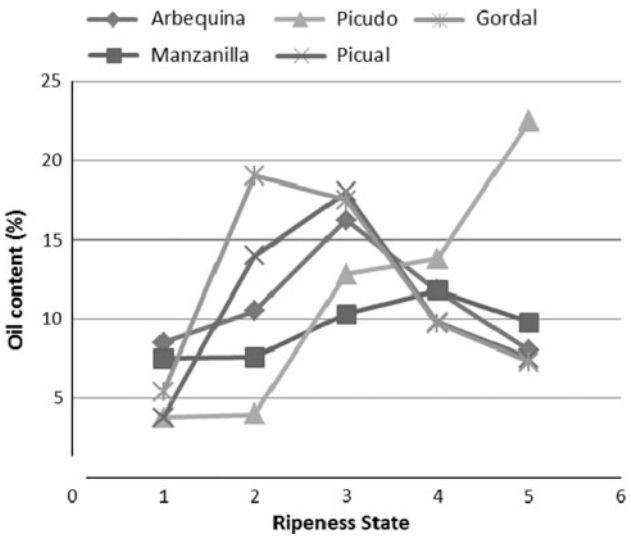

Fig. 1 Oil content, expressed as percentage, from olive fruit of each cultivar as a function of ripeness

Finding a unique explanation for the fluctuations in the fatty acid classes in the studied samples was not an easy task. The synthesis of certain fatty acid classes during the different ripening stages, the fatty acid dilution effect, the conversion between different classes by means of specific enzymes or the antioxidant composition of the olive drupes can explain these fluctuations.

The unhealthy properties of *trans* fatty acids, the contents of which determines oil quality [22], are well known. The concentration of *trans* fatty acids in the oil obtained from the drupes at different ripeness states ranged between 0.3 and 5.8% for gordal and arbequina cultivars, respectively. There is no information in the literature about the evolution of *trans* fatty acids with ripeness state, but the concentrations obtained for the different cultivars at the ripeness state number 5 are in agreement with previous research [18]. Figure 2a shows the *trans* fatty acid percentage in the oil extracted from the whole fruit of each cultivar as a function of the ripeness state. As can be seen in the figure, two trends were observed. A W-shaped trend was found for the arbequina, picual and picudo cultivars. In the case of picudo and arbequina cultivars, the concentration at the ripeness state 4 was higher than in the ripeness state 5 (modified W shape). The opposite trend was found for picual cultivar (real W shape). The other trend, which shows a V shape, was found in the remaining cultivars. The minimum *trans* fatty acid percentages were found for the

Table 1 Percentage of *trans*, saturated, monounsaturated, polyunsaturated, ω3 and ω6 fatty acids in five ripeness states of different cultivars of olive drupes

Cultivar	Ripeness state	*Trans*-fatty acids	Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids	ω3 Fatty acids	ω6 Fatty acids
Arbequina	1	6.18	15.38	66.03	18.58	0.00	2.27
	2	4.79	10.64	45.57	43.79	0.00	0.87
	3	5.83	8.35	41.87	49.78	0.00	0.26
	4	3.44	10.10	44.28	45.63	0.00	0.58
	5	3.17	10.37	44.52	45.10	0.00	0.08
Gordal	1	1.65	14.22	77.24	8.54	0.00	0.00
	2	0.29	15.50	74.77	9.72	0.27	0.26
	3	0.52	14.74	75.75	9.51	0.27	0.00
	4	1.28	13.52	69.26	17.21	0.09	0.43
	5	1.45	12.51	80.87	6.62	0.00	0.07
Manzanilla	1	3.91	18.80	74.29	6.91	0.13	0.00
	2	0.72	19.49	69.48	11.03	3.98	0.00
	3	0.66	21.39	56.69	21.93	9.60	0.00
	4	0.94	17.73	59.27	23.01	10.87	0.00
	5	1.31	19.79	66.52	13.70	0.00	0.00
Picual	1	2.21	9.21	77.96	12.83	0.00	0.00
	2	1.15	18.98	65.38	15.64	1.19	0.00
	3	1.63	13.33	64.70	21.97	0.70	0.17
	4	1.09	17.51	66.80	15.69	1.14	0.65
	5	2.16	14.24	59.76	25.99	0.00	0.39
Picudo	1	1.02	12.55	73.59	13.86	5.60	0.52
	2	3.36	13.15	75.23	11.62	6.05	0.33
	3	3.03	11.91	79.27	8.83	5.55	0.17
	4	2.39	13.29	78.88	7.83	0.03	0.10
	5	1.80	13.52	84.14	2.34	0.21	0.00

oil extracted at ripeness state 2. These behaviours can be explained by the binomial extraction method/antioxidant compounds, which favour and hinder, respectively, the *cis*/*trans* isomerisation of the fatty acids [22]. The percentage of saturated fatty acids present in the different cultivars ranged between 8.3 and 21.4%, for arbequina and manzanilla cultivars, respectively. In general, these results are in agreement with those found in the literature [18, 23]. Figure 2b shows the percentage of saturated fatty acids in the oil extracted from each cultivar. There is no clear trend in the variation of these fatty acids with ripeness. At least four different behaviours can be distinguished in the tested cultivars. N, M and Δ shape behaviours were observed for manzanilla, picual and gordal cultivars, respectively. The maximum values were obtained at the ripeness state 3 and 5 (N shape); 2 and 4 (M shape); and 3 (Δ shape) for manzanilla, picual and gordal cultivars, respectively. In the case of arbequina and picudo, both had the same behaviour: V shape with the minimum value at ripeness state 3. These results are in agreement with those found in the literature for typical Moroccan cultivars [24].

One of the main characteristics of olive oil is the presence of a high amount of monounsaturated fatty acids, endowed with healthy properties. Therefore, their presence is characteristic of high-quality oil. The monounsaturated fatty acid percentage obtained from the different cultivars and ripeness states ranged between 41.9% and 84.1% for arbequina and picudo cultivars, respectively. Exceptionally low values for these acids were obtained in arbequina drupes in comparison with the rest of cultivars (see Fig. 2c). These values are in agreement with those found in the literature for drupes from the same cultivars [18, 23, 25]. Figure 2c shows the percentage of monounsaturated fatty acids present in the oil extracted from drupes of different cultivars and ripeness states. In this case, three trends were observed. Trend one was that the percentage of monounsaturated fatty acids increased with the ripeness state, as observed in the picudo cultivar. This behaviour was also found in the literature for Chetoui and Picholine cultivars [26, 27], which are the most common cultivars in Tunisia and Morocco, respectively. Thus, some taxonomical relationship between the three cultivars could exist.

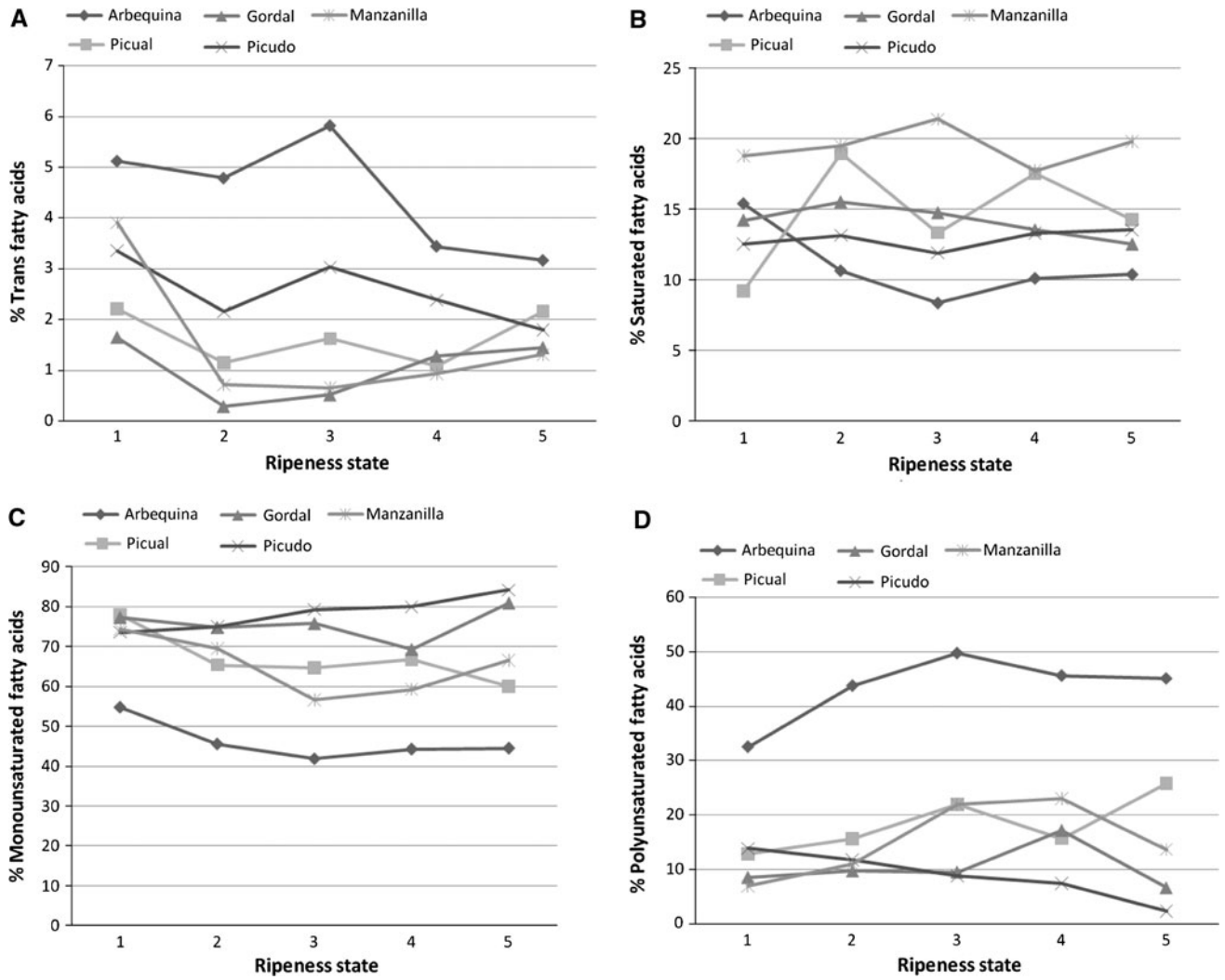

Fig. 2a–d Fatty acid percentage of the oil extracted from each cultivar of olive fruit as a function of ripeness. **a** *trans*-fatty acids, **b** saturated fatty acids, **c** monounsaturated fatty, **d** polyunsaturated fatty acid

The second trend was found for the picual cultivar, in which the percentage of monounsaturated fatty acids underwent a rapid decrease between ripeness states 1 and 2, and then decreased slightly until the end of ripening. This behaviour was found previously by Gutiérrez et al. [23] for the same cultivar. Finally, a V shape behaviour was found for the rest of the cultivars. The minimum percentage of monounsaturated fatty acids belonged to ripeness state 3 or 4, as a function of the cultivar (Fig. 2c). These results were similar to those provided by Sakouhi et al. [24] for different Tunisian cultivars.

The concentration of polyunsaturated fatty acids is a key parameter to study oil quality. The presence of these fatty acids in high concentration favours thermal degradation of oils as they can be oxidized more easily than those with low polyunsaturated fatty acid contents. The percentage of polyunsaturated fatty acids obtained from the different cultivars and ripeness states ranged between 2.3 and 49.7% for picudo and arbequina, respectively. Exceptionally high values, from two to four times higher, were obtained for arbequina drupes in comparison with the rest of cultivars (Fig. 2d). Similar results were found by Gómez et al. [18] for this cultivar at ripeness state 5. The remaining values are in agreement with the information found in the literature for different cultivars harvested in the Calabrian area [28].

Figure 2d shows the percentage of polyunsaturated fatty acids present in the oil extracted from drupes of different cultivars and ripeness states. Once again, three trends were observed. In the first, found for the picudo cultivar, the percentage of polyunsaturated fatty acids decreased with the ripeness state. This trend was also reported by Poiana and Mincione [28] for cassanese cultivars, which is a typical cultivar in Italy. The second trend was found for the picual cultivar; in this case, an N-shaped trend, with maximal concentrations at ripeness states 3 and 5. Similar

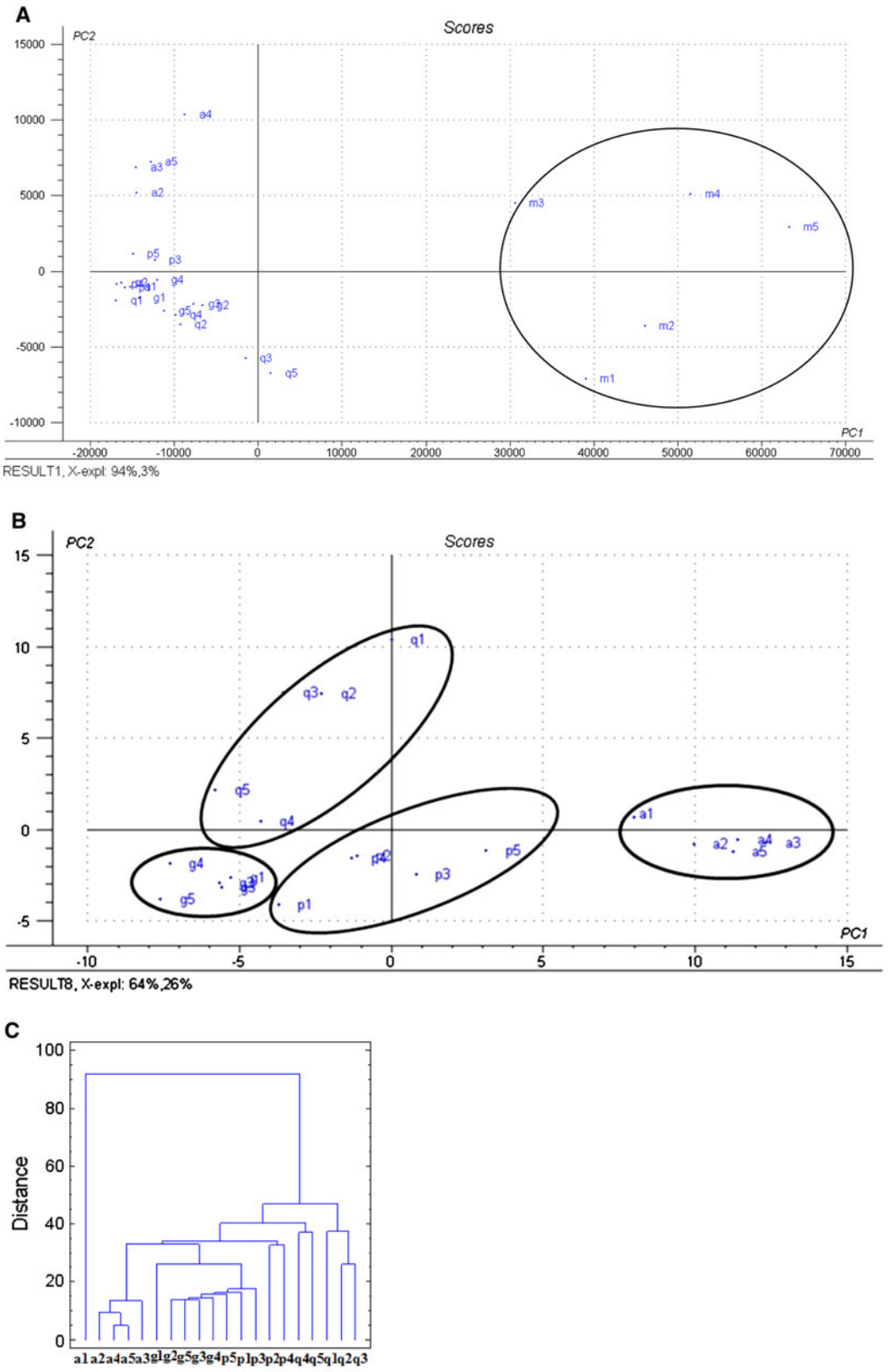

Fig. 3 **a** Principal component analysis (PCA) model obtained for the different cultivars and ripeness states as raw variables. **b** PCA model obtained for the different cultivars and ripeness states, manzanilla not included. **c** Hieratical cluster analysis of four olive cultivars. *a* Arbequina, *q* picudo, *p* picual, *g* gordal, *m* manzanilla. Numbers from *1* to *5* represent the ripening state

results were found in the literature for coratina cultivar drupes, which is usual in Italy [28]. Finally, a Δ-shaped trend was found for the rest of the cultivars. As can be seen in Fig. 2d, the maximum percentage of polyunsaturated fatty acids is achieved at ripeness state 3 or 4, as a function of the cultivar. This behaviour is the most common, and was found for a wide range of cultivars, including the most usual in Tunisia, Morocco or Italy [24, 27, 28].

The evolution of individual fatty acids with ripeness was also studied. The results of this analysis can be seen in the electronic supplementary material. The results discussed in this section are the weighted sum of the individual behaviours.

Statistical Analysis

PCA and HCA were used to determine the influence of the olive-tree cultivar and the harvest period on the fatty acid profile. These methods were used as the small number of olive samples used for development of the models prevented the use of more sophisticated supervised pattern recognition techniques, such as K-Nearest Neighbour (KNN) or Soft Independent Modelling of Class Analogy (SIMCA). However, PCA and HCA are useful tools with which to visualize differences between samples.

As a first step in the statistical analysis, a PCA model was developed using the individual fatty acid concentrations (26 compounds) obtained for the different cultivars and ripeness states (25 samples) as raw variables. No data treatment was used. The results of this model can be seen in Fig. 3a. The samples appear to be grouped as a function of the cultivar, but this conclusion cannot be obtained from the figure. Manzanilla drupes, at different ripeness states, are clearly different from the rest of the samples. The explained variance is 97% for two principal components and the analytes with a higher influence on the development of the model are those present at the highest concentrations in the samples: C18:1 n9, C16:0, C18:2 cc or C20:4.

In step 2, manzanilla samples were removed from the model to study the trend for the other cultivars. Both, raw and pretreated (normalised and differentiated) data were tested to obtain the best separation between the tested samples. The best results were obtained using pretreated data as variables (Fig. 3b). In this figure, the different samples were grouped as a function of the cultivar. Ellipses have been used to group samples from the same cultivar. Analytes that exert a higher influence in the development of the model are the same as in the previous model, in addition to C18:1 n7. Two principal components were necessary to explain 90% of the data variability.

Hieratical cluster analysis (HCA) was also developed. As can be seen in Fig. 3c, samples were grouped as a function of the cultivar and not as a function of the ripeness state. As additional information, it can be said that samples collected at ripeness state 1 are different from the rest of the samples of the same cultivar. Furthermore, arbequina samples collected at ripeness state 1 are completely different from the rest of the samples, with an Euclidean squared distance of 92. This distance is very small in the case of picual and picudo cultivars (smaller than 20). This means that the oils obtained from gordal and picual are similar, but it is possible to find differences between both cultivars. This result is in agreement with those from the PCA model (Fig. 3b).

Similar PCA models were obtained by Orozco-Solano et al. [29] using the concentration of the individual fatty alcohols and sterols present in olive drupes. In the same research, normalized and differentiated data were also used for the development of PCA models, which revealed that the olive-drupe samples can be grouped as a function of the ripeness state. This result cannot be obtained using the fatty acid profile as variables for development of the models.

Monovarietal PCA models were developed to study the influence of ripening on the oil obtained from each cultivar. The results of this experiment can be found as electronic supplementary material. In all cases, the oil obtained at ripeness state 5 was different from the rest. In addition, for most of the cultivars with the exception of manzanilla, the oil obtained at ripeness state 1 was also different from the rest. In the case of the manzanilla cultivar, the oils obtained at ripeness states 1, 2 and 4 were similar and different from that obtained at ripeness states 3 and 5. Therefore, no significant differences exist in the fatty acid profile of the oils obtained from drupes at the ripeness states ranging between 2 and 4.

Conclusions

The different trends observed for the fatty acid classes, i.e. saturated, monounsaturated, polyunsaturated and *trans* fatty acids, can be used for the selection of the optimal ripening state. The unexpected trend obtained for the *trans* fatty acids family can be explained by the alteration of the fatty acid profile produced by the extraction method.

Statistical studies reveal that manzanilla drupes, at different ripeness states, are clearly different from the rest of the samples. In addition, there are no significant differences in the fatty acid profile of the oils obtained from drupes at ripeness states ranging between 2 and 4.

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References

1. Conde C, Delrot S, Gerós H (2008) Physiological, biochemical and molecular changes occurring during olive development and ripening. J Plant Physiol 165:1545–1562
2. Matos LC, Pereira JA, Andrade BP, Seabra MR, Beatriz M, Oliveira PP (2007) Evaluation of a numerical method to predict the polyphenols content in monovarietal olive oils. Food Chem 102:976–983
3. Bandelj D, Jakše J, Javornik B (2002) DNA fingerprinting of olive varieties by microsatellite markers. Food Technol Biotechnol 40:185–190
4. Morello JR, Romero MP, Motilva MJ (2004) Effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga, and Morrut Cultivars. J Agric Food Chem 52:6002–6009
5. Kiritsakis A, Christie WW (2000) Analysis of edible oils. In: Harwood J, Aparicio R (eds) Handbook of olive oil: analysis and properties. Aspen, Gaithersburg, pp 129–158
6. Visioli F, Galli C (2002) Biological properties of olive oil phytochemicals. Crit Rev Food Sci Nutr 42:209–221
7. Aguilera MP, Beltran G, Ortega D, Fernandez A, Jimenez A, Uceda M (2005) Characterisation of virgin olive oil of Italian olive cultivars: Frantoio and Leccino, grown in Andalusia. Food Chem 89:387–391
8. Tsimidou M, Karakostas KX (1993) Geographical classification of Greek virgin olive oil by non-parametric multivariate evaluation of fatty acid composition. J Sci Food Agric 62:253–257
9. Panelli G, Montedoro GF (1988) Scelte varietali, condizioni pedoclimatiche, maturazione del frutto e caratteristiche qualitative dell'olio di oliva. Conference Proceeding "Aspetti fisiologici della cascola, della maturazione, della conservazione e della trasformazione post-raccolta dei frutti". Turin (Italy) 3–4 October, pp 99–105
10. Alonso V, Aparicio R (1993) Characterization of European virgin olive oils using fatty acids. Grasas y aceites 44:18–24
11. Criado MN, Motilva MJ, Goni M, Romero MP (2005) Comparative study of the effect of maturation process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from Arbequina and Farga cultivars. Food Chem 100:748–755
12. Baccouri B, Zarrouk W, Khichene D, Nouari I, Ben Youssef N, Daoud D (2007) Influence of fruit ripening and crop yield on chemical properties of virgin olive oils from seven selected oleasters (*Olea europea* L.). J Agron 6:388–396
13. Beltrán G, Aguilera MP, Del Rio C, Sanchez S, Martinez L (2005) Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. Food Chem 89:207–215
14. Barranco D, de Toro C, Rallo L (1998) Épocas de maduración de cultivares de olivo en Córdoba. Invest Agr: Prod Prot Veg 13:359–368
15. International olive oil council, Method of analysis, COI/T.20/Doc.no.24, 2001. http://www.internationaloliveoil.org/downloads/orga6.pdf
16. Sánchez-Ávila N, Mata Granados JM, Ruiz-Jiménez J, Luque de Castro MD (2009) Fast, sensitive and highly discriminate gas chromatography–mass spectrometry method for profiling analysis of fatty acids in serum. J Chromatogr A 1216:6864–6872
17. Barrueta LA, Alonso-Salces RM, Héberger K (2007) Supervised pattern recognition in food analysis. J Chromatogr A 1158:196–214
18. Gómez-González S, Ruiz-Jiménez J, Luque de Castro MD (2010) Fatty acid profiling of the main tissues of spanish olive fruit: effect of the extraction method. J Am Oil Chem Soc 87:1413–1423
19. Nergiz C, Ergönül PG (2009) Organic acid content and composition of the olive fruits during ripening and its relationship with oil and sugar. Sci Hortic 122:216–220
20. Nergiz C, Engez Y (2000) Compositional variation of olive fruit during ripening. Food Chem 69:55–59
21. Sánchez J (1994) Lipid photosynthesis in olive fruit. Prog Lipid Res 33:97–104
22. Gamel TH, Kiritsakis A, Petrakis Ch (1999) Effect of phenolic extracts on trans fatty acid formation during frying. Grasas y Aceites 50:421–425
23. Gutiérrez F, Jiménez B, Ruíz A, Albi MA (1999) Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties picual and hojiblanca and on the different components involved. J Agric Food Chem 47:121–127
24. Sakouhi F, Harrabi S, Absalon C, Sbei K, Boukhchina S, Kallel H (2008) α-Tocopherol and fatty acids contents of some Tunisian table olives (*Olea europea* L.): changes in their composition during ripening and processing. Food Chem 108:833–839
25. López-López A, Rodríguez-Gómez F, Cortés-Delgado A, Montaño A, Garrido-Fernandez A (2009) Influence of ripe table olive processing on oil characteristics and composition as determined by chemometrics. J Agric Food Chem 57:8973–8981
26. Ajana H, El Antari A, Hafidi A (1998) Fatty acids and sterols evolution during the ripening of olives from the Moroccan Picholine cultivar. Grasas y Aceites 49:405–410
27. Zarrouk W, Haddada FM, Baccouri B, Oueslati I, Taamalli W, Fernandez X, Lizzani-Cuvelier L, Daoud D, Zarrouk M (2008) Characterization of virgin olive oil from Southern Tunisia. Eur J Lipid Sci Technol 110:81–88
28. Poiana M, Mincione A (2004) Fatty acids evolution and composition of olive oils extracted from different olive cultivars grown in Calabrian area. Grasas y Aceites 55:282–290
29. Orozco-Solano M, Ruiz-Jiménez J, Luque de Castro MD (2010) Characterization of fatty alcohol and sterol fractions in olive tree. J Agric Food Chem 58:7539–7546