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Effect of the Maturation Process of the Olive Fruit on the Phenolic Fraction of Drupes and Oils from Arbequina, Farga, and Morrut Cultivars

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The purpose of the work was to investigate the effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga, and Morrut cultivars. The level in the phenolic content of olive drupes declines rapidly during the black maturation phase. A general decreasing trend was observed too in the phenolic content of olive oils during the ripening process in the three varieties studied. Important differences in the high-performance liquid chromatography profile between varieties were observed. These included the presence of very low amounts of lignans in olive oils proceeding from the Morrut cultivar, and the presence of three peaks after elution of 3,4-DHPEA-EDA in the Farga and Morrut cultivars, which could be used as differentiating parameters. Sensory profile differences were observed between olive cultivars and due to the ripening process.

KEYWORDS: Olive fruit; virgin olive oil; phenolic compounds

INTRODUCTION

Phenolic compounds are secondary plant metabolites that are biosynthesized through the shikimic acid pathway. The polyphenolic profile differs between varieties of plants of the same species. Polyphenolic compounds are ubiquitous in all plant organs and are, therefore, an integral part of the human diet. Recent interest in food phenolics has increased greatly because of the antioxidant and free radical scavenging abilities associated with some phenolics and their potential effects on human health (*1*). The olive (*Olea europaea* L.) is a source of several phenolic compounds with important antioxidant properties (*2*). Oleuropein, demethyloleuropein, ligstroside, and oleoside represent the predominant phenolic oleosides (*3*), whereas verbascoside is the main hydroxycinnamic derivative of the olive fruit (*4*).

The phenolic compounds content is an important parameter in the evaluation of virgin olive oil quality given that phenols contribute to oil flavor and aroma and protect it from oxidation through their free radical scavenging and metal chelating properties (*5*, *6*). Because the phenolic content of olive drupes can be strongly affected by agronomic parameters such as cultivar, pedoclimatic production conditions, agronomic techniques, and fruit ripening, its content in the oil can also be conditioned by those factors.

Olive ripening lasts several months and varies according to the growing area, variety, water availability, temperature, and farming practices. Studies of changes in the phenolic profile and content related to maturation have largely been focused on the olive fruit and particularly on changes in the oleuropein content. Oleuropein is the major phenolic compound in the pulp of many cultivars in which its concentration reaches relatively high levels in immature olive fruit during the growth phase (*2*). However, its concentration declines with the physiological development of the fruit (*7*) in what is termed the green maturation phase and this may be correlated to the increased activity of the hydrolytic enzymes with maturation (*1*−*8*). The level continues to decline rapidly during the black maturation phase characterized by the appearance of anthocyanins (*9*).

These changes in drupes are directly reflected in the composition of the olive oil since virgin olive oil is obtained by mechanical or physical methods under conditions, especially heat, guaranteed to avoid any alteration to the oil. However, as a consequence of cellular destruction and the mixing of cellular content during olive oil extraction (crushing and malaxation), many modifications take place in olive compounds. These include hydrolysis of glycerides by lipases, hydrolysis of glycosides and oligosaccharides by glucosidases, oxidation of phenolic compounds by phenoloxidases, and polymerization of free phenols (*10*). During crushing, secoiridoid aglycons such as 3,4-DHPEA-EDA, *p*-HPEA-EDA, and 3,4-DHPEA-EA can be produced by the hydrolysis of oleuropein, demethyloleuropein, and ligstroside. Besides secoiridoid aglycons, virgin olive oils contain several compounds such as phenolic acids (caffeic, vanillic, and *p*-coumaric), phenolic alcohols (3,4-DHPEA and *p*-HPEA), lignans (acetoxypinoresinol and pinoresinol), and flavonoids (luteolin and apigenin).

Although interest in phenolic compounds is related primarily to their antioxidant activity, which improves virgin olive oil oxidative stability and extends olive oil shelf life, they also show important biological activity in vivo and may contribute to

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combating diseases related to oxygen radical formation when this exceeds the antioxidant defense capacity of the human body. With regard to the organoleptic characteristics of virgin olive oil, sensory attributes such as bitter and pungent are correlated with the total phenol concentration (*11*, *12*).

The phenolic content of plants depends both quantitatively and qualitatively on their genetic make up. There are studies showing differences in the phenolic content of olive fruit and virgin oils from different Italian (*8*) and Spanish olive cultivars (*13*). These can be used to establish taxonomic affinities and/or differences.

Environmental factors and agronomic practices, such as irrigation, affect the phenolic composition of virgin olive oil. Thus, previous studies carried out by our research group showed that the application of different irrigation strategies on olive trees (Arbequina cultvar) (*14*, *15*) and freeze injuries in olive fruit during the harvest period (*16*) affected the phenolic profiles of virgin olive oil and as a consequence the oxidative stability and some sensory attributes related to these.

The purpose of the current work was to investigate the effect of the maturation process of the olive fruit on the phenolic fraction of drupes and oils from Arbequina, Farga, and Morrut cultivars (the most commonly grown in the Ebro Valley, Spain). Quantitative and qualitative differences in phenolic compounds were also studied in order to determine if phenolic compounds could be used as varietal identificators. Moreover, an organoleptic assessment of the olive oils extracted was carried out with the aim of defining the sensorial profile of the olive cultivars studied and the effects of maturation on olive oil attributes.

MATERIALS AND METHODS

Plant Material. The trial was carried out during the 2002 crop season in three different olive groves (*O. europaea* L.) on Arbequina, Farga, and Morrut cultivars located in the Ebro Valley in Spain. The general climate of this area is moderate, common to the Mediterranean region.

At sampling, which started in the second week of September (when maturation starts) and finished in the second week of December (at harvest), representative samples from each one of the three cultivars were picked and put into 3 kg boxes and taken directly to the pilot plant where they were processed.

Ripening Index. Ripeness was determined according to the guidelines of the Spanish National Institute of Agronomic Research based on a subjective evaluation of the olive skin and pulp colors (*17*). The procedure consists of distributing a randomly taken sample of 100 fruit into eight groups: intense green (group $N = 0$), yellowish green (group $N = 1$), green with reddish spots (group $N = 2$), reddish-brown (group $N = 3$), black with white flesh (group $N = 4$), black with 50% purple flesh (group $N = 5$), black with 50% purple flesh (group $N = 6$), and black with 100% purple flesh (group $N = 7$). The index is expressed as $\sum(N_i n_i)/100$ where N is the group number, and n is the fruit number in that group. The ripeness index (RI) values range from 0 to 7.

Oil Extraction. An Abencor analyzer (MC2 Ingenierías y Sistemas, Seville, Spain) was used to process the olives in the pilot extraction plant. The unit consists of three essential elements: the mill, the thermobeater, and the pulp centrifuge. After it was processed in the mill, the oil was separated by decanting, transferred into dark glass bottles, and stored in the dark at 4 °C. The oil yield of the olives was expressed as a percentage of dry weight.

Olive Analyses. *Moisture Content.* Samples of approximately 10 g of olive flesh from 25 olives were weighed and then dried for 24 h at 105 °C, cooled for 30 min in a desiccator, and reweighed, according UNE standard Spanish method (*18*).

Oil Content. Dried samples of olive flesh (approximately 4 g) were measured with a NMS 100 Minispec NMR Analyzer (Bruker Analytik, Silberstreifen, Germany) using the software ExpSpel Version 2.10 software.

Extraction and Quantification of Phenolic Compounds. The methodology for extracting the phenolic compounds followed an adaptation of the method of Fantozzi and Montedoro (*19*) as reported by Chimi and Atouati (*20*). Approximately 1 g of ground olive flesh from 25 olives was mixed in duplicate with 40 mL of hexane and agitated for 4 min. The upper phase was recovered, and the extraction was repeated twice with the lower phase to allow removal of pigments and most of the lipids. The phenolic compounds were extracted with 80 mL of 80% (v/v) methanol containing 400 ppm of sodium metabisulfite. The mixture was homogenized for 30 s using a Polytron homogenizer to separate the hydromethanolic phase. This procedure was repeated twice. The hydromethanolic phases were combined and filtered with 0.45 μm nylon syringe filter.

HPLC Analysis of Phenolic Compounds. The HPLC system consisted of a Waters 717 plus Autosampler, a Waters 600 pump, a Waters column heater module, and a Waters 996 photodiode array detector controlled by a Empower software (Waters Inc., Milford, MA).

The phenolic fraction extracted was dissolved in 1 mL of methanol and analyzed by high-performance liquid chromatography (HPLC). The column was a Inertsil ODS-3 (5 μm, 15 cm $\times$ 4.6 mm i.d., GL Sciences Inc.) equipped with a Spherisorb S5 ODS-2 (5 μm, 1 cm $\times$ 4.6 mm i.d., Technokroma, Barcelona, Spain) precolumn. HPLC analysis was performed following the same procedure as Montedoro et al. (*21*). The eluents were 0.2% acetic acid (pH 3.1) and methanol, the flow rate was 1.5 mL/min, and the injection volume was 20 μL. The total run time was 60 min, the initial composition was 95% acetic acid and 0.2 and 5% methanol, and the gradient changed as follows. The concentration of methanol was maintained for 2 min, then it was increased to 25% in 8 min, and finally, the methanol percentage was increased to 40, 50, and 100% in 10 min periods. It was maintained at 100% for 5 min. Initial conditions were reached in 15 min. Chromatograms were recorded at 278 and 339 nm. Individual phenols are expressed as mg per kg of dry weight olive pulp (mg kg^{-1} dw).

Olive Oil Analyses. Stability is expressed as the oxidation induction time (h) measured with a Rancimat 679 apparatus (Metrohm Co., Switzerland) using an oil sample of 3 g warmed to 120 °C and 20 L h^{-1} air flow. The time taken to reach a fixed level of conductivity was measured (*22*).

The bitter index (K_{225}) was evaluated by the extraction of the bitter components of a sample of 1.0 ± 0.01 g oil dissolved in 4 mL of hexane passed over a C18 column (Waters Sep-Pack Cartridges), previously activated with methanol (6 mL) and washed with hexane (6 mL). After elution, 10 mL of hexane was passed to eliminate the fat, and then, the retained compounds were eluted with methanol/water (1/1) to 25 mL. The absorbance of the extract was measured at 225 nm against methanol/water (1/1) in a 1 cm cell (*11*).

The total phenol content was analyzed using the modified isolation method described by Vazquez-Roncero et al. (*23*) with triple extraction of an oil-in-hexane solution with a 60% vol/vol water/methanol mixture. The total concentration of phenols was estimated with Folin−Ciocalteau reagent at 725 nm. The results were expressed as mg of caffeic acid per kg of oil (mg caffeic acid kg^{-1}).

The organoleptic evaluation of the oil was carried out according to the Official European Methods of Analysis by panellists of Les Garrigues Protected Designation of Origin (PDO). The panel consisted of 10 trained tasters who carried out a description of the oil flavor and quality grading. In this paper, only the bitter, pungent, and sweet sensory attributes are reported. The descriptive analysis used a six point intensity ordinal rate scaling from 0 (no perception) to 5 (extreme) to quantify the intensity of sensory attributes.

Analysis of Phenolic Compounds. *Phenolic Extraction.* Olive phenols were extracted following the procedure reported above in Olive Analyses. Oil phenols were extracted from virgin olive oil following the procedure reported by Tovar et al. (*14*).

The phenolic extract was rotaevaporated and dissolved in 1 mL of methanol and analyzed using HPLC. Chromatographic conditions were the same as those used with the olive pulp phenolic fraction.

Reference Compounds. Oleuropein, verbascoside, apigenin, luteolin, tyrosol, and *p*-coumaric acid were obtained from Extrasynthèse Co. (Genay, France). Vanillic acid, vanillin, and ferulic acid were obtained from Fluka Co. (Buchs, Switzerland). Hydroxytyrosol was kindly

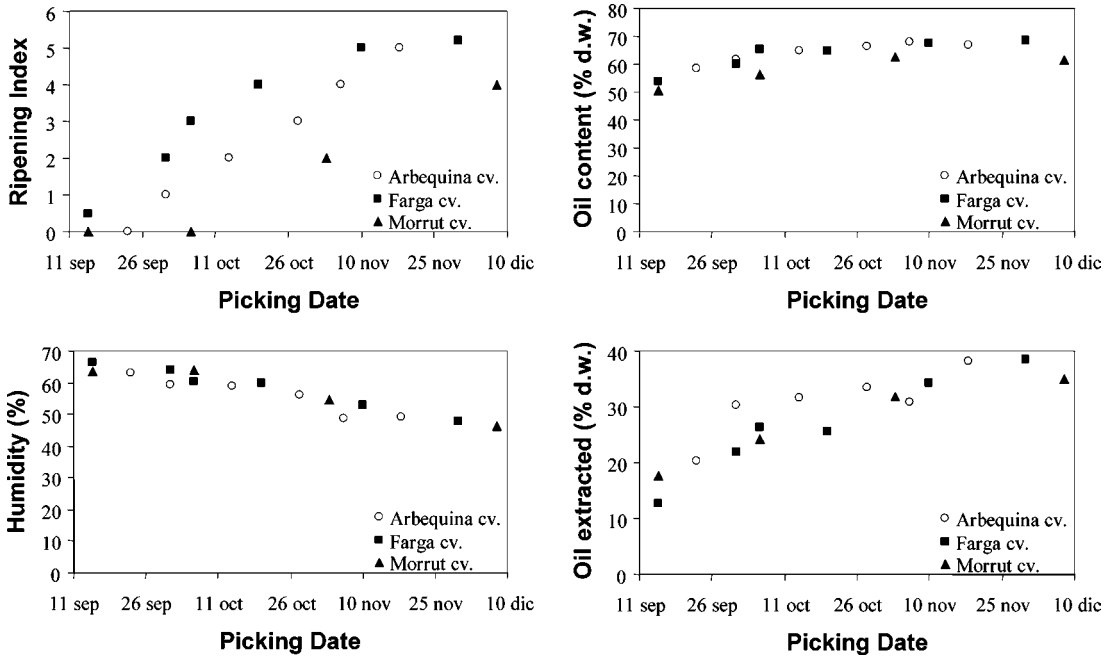

Figure 1. Effect of picking date on olive drupes of Arbequina cv., Farga cv., and Morrut cv. harvested at different ripening indexes. Values are means of four independent values, and the standard error is lower than 5%.

donated by Professor Montedoro (University of Perugia, Italy). The rest of the phenolic compounds were obtained using a semipreparative HPLC column Spherisorb ODS-2 (5 μm, 25 cm $\times$ 10 mm i.d., Technokroma) and a flow rate of 4 mL/min. The mobile phases and gradient are described elsewhere (*14*). Individual phenols were quantified by a four point regression curve on the basis of the standards obtained from commercial suppliers or from preparative HPLC as described above. Quantification of the phenolic compounds was carried out at 280 nm. Phenols are expressed as mg per kg of oil (mg kg^{-1}).

Mass Spectrometry. The mass spectra of selected (purified) compounds were performed on a micromass ZMD (Waters Inc.) equipped with an electrospray ionization ion source (ESI). The ion spray mass spectra in the negative ion mode were obtained under the following conditions: capillary voltage, 2.5 kV; cone voltage, 10 V; desolvation temperature, 400 °C; and source temperature, 120 °C.

Statistical Analysis. The data were subjected to an analysis of variance using the SAS version 8.02 (SAS Institute Inc., Cary, NC). Separation of the means was obtained through the least-squares means test, and the significant difference was defined at $p \leq 0.05$.

RESULTS

The Arbequina variety is considered rustic with good resistance to frosts and highly adaptable to different climates and soils. The fruit is small with a rounded top, and it is black at maturation, which occurs in midseason (the second half of November) but not all at once. The oil yield is good and of excellent quality with good organoleptic characteristics. The Farga is a vigorous, traditional variety. The fruit is medium-sized, elongated, and slightly asymmetric in shape. This variety has a good quality, high oil content, but this is difficult to extract. The Morrut is a vigorous variety, producing on alternate years, and it is slow to come into production. Maturation is very late, the oil production is medium, and the oil is of low stability.

Figure 1 shows the effect of the picking date on olive drupes of Arbequina, Farga, and Morrut cultivars harvested at different RIs. The Farga cultivar was the earliest variety to start black maturation reaching RI values around 5−6 in the first week of November. On the other hand, the Arbequina cultivar started maturation later but the RI increased more linearly through the time, although olive ripening was not synchronous over the tree. Finally, the Morrut cultivar started the black maturation during October reaching RI values of 4 in the first week of December. Color changes are associated with the decline in chlorophyll and oleuropein levels and appearance of anthocyanins (*2*). The phase of fruit development referred to as black maturation is a direct result of a significant increase in the anthocyanin content. However, in olive fruit, the "technological ripening" corresponds to intermediate stages of maturation and not to very late stages of this (*10*). The oil content of the olives (expressed as % of dry weight) remained practically unchanged during the sampling period (**Figure 1**), and it seems more related to picking date than to the ripening stage of the fruit. In all cultivars studied, a slight rise was observed between the two first samplings (ripening index 0−1), which may be due to the fact that the triglyceride synthesis in the olive fruit was still not finished in the sampling carried out in the middle of September. The three olive cultivars studied showed similar oil contents throughout the picking period. Significant differences on oil yield (expressed as % of dry weight of olive) were observed in all varieties between the first and the latter samplings (**Figure 1**). Extraction yields showed a rising trend in all varieties independently of the cultivar and oil content, reaching values of extracted oil of 35−38%, which correspond to varieties with a high oil yield. These results coincide with those in Gutiérrez et al. (*24*) and Salvador et al. (*25*) who observed that olive maturity significantly affects the extraction yield. These differences in the trend of oil yield may be due to a slightly lower value of moisture content in olive drupes at the end of the sampling period (**Figure 1**). A negative correlation ($r = -0.875$) between oil yield and moisture content was found in all of the olive cultivars studied, which would explain the high values of oil extracted in the later samplings despite there being a similar oil content throughout the sampling period. The highest correlation was found in the Farga cultivar ($r = -0.939$). No differences between varieties were observed in oil yield during ripening.

Figure 2 shows the chromatographic profile of the phenolic compounds of olive drupe and olive oil from the Arbequina, Farga, and Morrut cultivars harvested at the first picking date (September) that corresponded to a RI next to 0 (**Figure 1**).

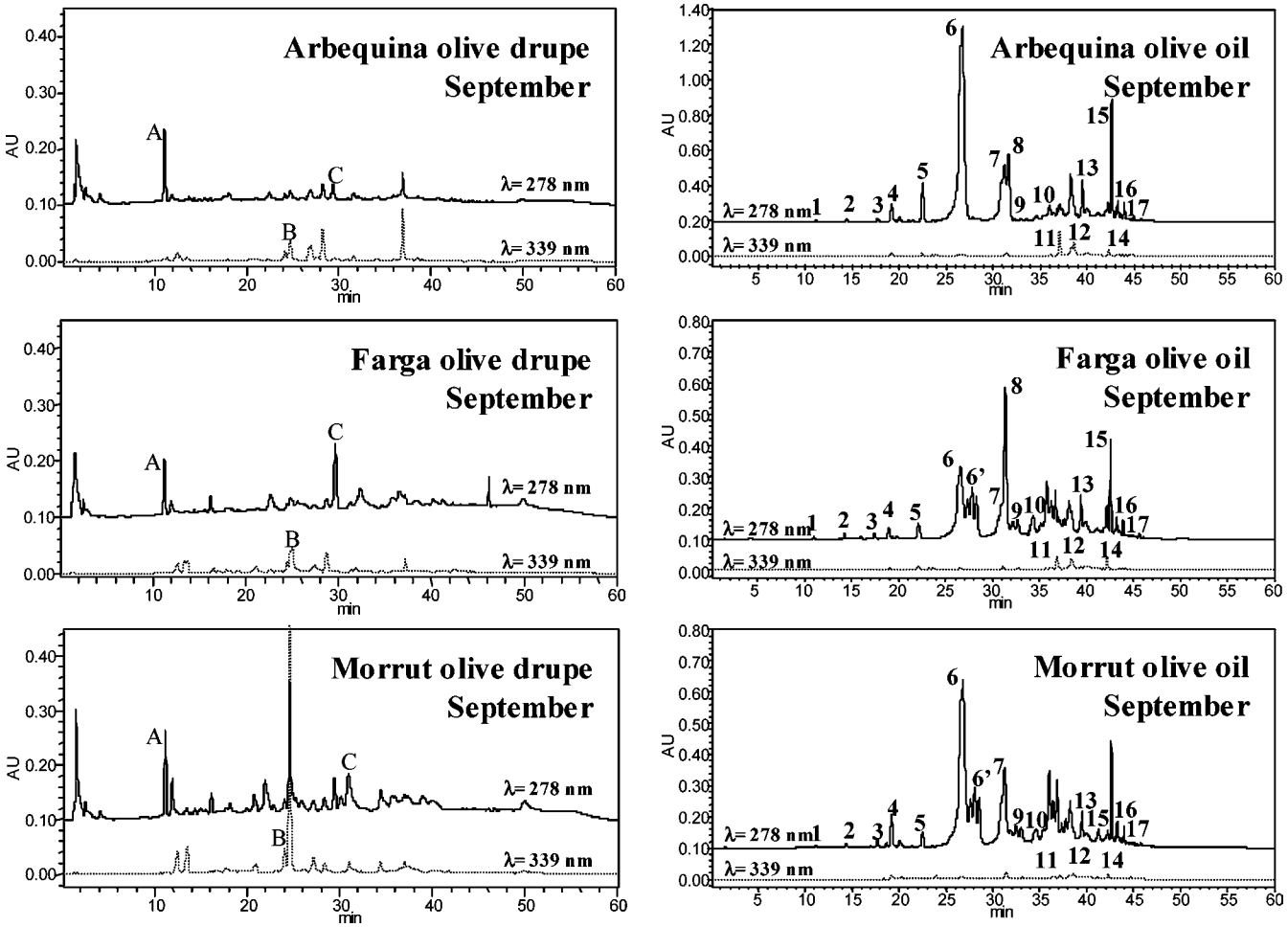

Figure 2. HPLC chromatograms (at 278 and 339 nm) of phenolic extracts from olive oil. See the Materials and Methods for chromatographic conditions. See **Table 1** to identify the peaks.

The most noticeable differences were the high levels of 3,4-DHPEA-EDA (peak 6) in oils from the Arbequina cultivar and the presence of three peaks (peak 6′) after 3,4-DHPEA-EDA in oils from the Farga and Morrut cultivars that corresponded to a structural isomer of 3,4-DHPEA-EDA according to the UV and MS spectra. That differentiated the Arbequina cultivar from the rest of cultivars studied in this trial. A similar compound was found in Arbequina olive oils stored for 1 year in darkness in the absence of air, but only one peak was reported and its UV and MS spectra coincided (*26*). In relation to the lignans fraction (peak 8), significant differences, which could be used as a varietal indicator, were observed between the Morrut and the other cultivars. Very low concentrations were found in that cultivar, while in the Arbequina and Farga cultivars it was one of the more abundant phenolic compounds in virgin olive oils. Finally, there were differences in the flavonoid fraction and the latter phenolic fraction values between the three cultivars studied in this trial. In this way, oils from the Morrut cultivar showed lower concentrations of all of the compounds that form that fraction.

Tables 1−**3** show the concentrations of the main phenolic compounds quantified in both fractions (olive pulp and olive oil) from the three cultivars, respectively. The main phenolic compounds in olive pulp in the three cultivars studied were hydroxytyrosol (3,4-DHPEA), verbascoside, and oleuropein. No data relating to olive pit are shown because low amounts of total phenolic compounds were quantified and no phenolic compounds could be identified. No significant differences were observed in the concentration of verbascoside on Farga and Arbequina cultivars, while on the Morrut cultivar very significant differences were observed between olive pulps from early and later picking dates. Moreover, the verbascoside content of Morrut olives with a low RI could be used as a varietal identification to permit discrimination between Morrut olives and the other varieties studied in this trial (**Figure 2**). The main phenolic compound observed in all cultivars was 3,4-DHPEA, which could be related to the degradation of oleuropein during maturation although its amount also decreases during the sampling period. Oleuropein declined with maturity in the fruit of the Farga cultivar (**Table 2**). In contrast, the oleuropein content of Arbequina and Morrut (**Tables 1** and **3**, respectively) fruits showed an increase during the first week of October (value RI of 1) before decreasing to very low or no detected concentrations in all varieties studied. This evolution is consistent with the findings of Amiot et al. (*27*) and Ryan et al. (*4*), who showed a slight initial decrease in the oleuropein content of Manzanillo cultivar fruit, which then increased by 50% when the fruits were green before decreasing again. In some instances, turnover may be simply related to recycling of phenolic moieties into new conjugates (*2*). This could explain the increase in oleuropein in Arbequina and Morrut cultivars shown in this study. On other occasions, more extensive degradation may be occurring, which appears to be the case with the decline in oleuropein levels accompanying fruit development (*2*). Both phenolic compounds (3,4-DHPEA and oleuropein) followed the same decreasing trend in all varieties

Table 1. Effect of the Picking Date in the Phenolic Compounds of Olive Pulp, Expressed as Dry Weight, and Virgin Olive Oils from Arbequina Cultivar and Their Effect in the Oil Parameters[a]

peak	phenolic compd[b] (mg kg^{-1})	picking date[c] 23th Sep	1st Oct	14th Oct	28th Oct	6th Nov	18th Nov	
				olive pulp				
A	3,4-DHPEA	8400	5300	2610	2540	2200	1580	**
B	verbascoside	500	780	870	220	370	660	NS
C	oleuropein	1710	3590	1140	1460	ND	ND	**
				olive oil				
1	3,4-DHPEA	0.60	0.48	0.54	0.42	0.55	0.56	NS
2	*p*-HPEA	1.49	1.15	1.03	0.92	1.02	1.16	**
3	vanillic acid	0.65	0.38	0.31	0.28	0.28	0.35	NS
4	vanillin	1.59	1.09	0.89	0.50	0.71	0.49	**
5	3,4-DHPEA-AC	126	112	230	203	247	208	**
6	3,4-DHPEA-EDA	1479	1633	1356	1528	1635	1278	NS
7	*p*-HPEA-EDA	148	129	106	92	109	84	**
8	lignans	102	74	81	72	70	68	*
9	*p*-HPEA-EA	3.53	3.19	2.74	2.41	2.56	1.49	**
10	3,4-DHPEA-EA	361	295	206	184	196	154	**
11	luteolin	2.07	3.13	1.56	1.25	1.08	0.83	**
12	apigenin	2.02	2.59	3.25	3.95	3.27	2.37	**
13	peak 13	222	161	130	97	120	105	**
14	flavonoid	1.93	1.30	1.56	1.25	1.08	0.83	**
15	peak 15	22	20	36	51	60	39	**
16	peak 16	4.59	3.58	6.83	7.27	6.84	4.73	**
17	peak 17	0.97	0.50	0.60	0.38	0.47	0.39	*
				parameter				
	total phenol (mg kg^{-1})	468	565	402	439	451	398	**
	α-tocopherol (mg kg^{-1})	223	197	166	159	152	144	*
	oxidative stability (h)	25.3	26.5	25.1	25.0	21.7	20.8	**
	K_{225}	0.497	0.558	0.476	0.417	0.426	0.395	**

[a] ND, not detected. [b] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycon. [c] Significance level. NS, not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$.

sampled as probably a consequence of hydrolysis and oxidation processes occurred during olive drupe maturation as reported by Fedeli et al. (*28*).

The results from the olive oil are mainly conditioned by the composition of the olive drupes and also by the extraction process, which was the same for all of the samples (Abencor system). Consequently, changes is this study could be attributed to olive drupe composition. The HPLC phenolic profile of virgin olive oils studied in this trial was characterized by five main fractions: simple phenols, secoiridoid derivatives, lignans, flavonoids, and the latter part of the chromatogram (**Figure 2**). DHPEA-AC and secoiridoid derivatives (3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA, and 3,4-DHPEA-EA) were the major representative compounds of the phenolic fraction of virgin olive oils and were oleuropein-related products present in olive drupes.

The concentration of the quantified phenolic compounds in virgin olive oils from the three varieties studied is reported in **Tables 1**−**3**. Simple phenols, 3,4-DHPEA, *p*-HPEA, vanillin, and vanillic acid, were present in very low concentrations, and a decreasing trend during the maturation process was observed in the vanillin and vanillic acid concentrations of the oils. The concentrations of all secoiridoid derivatives of Arbequina olive oils (**Table 1**) suffered a decrease during maturation with the exception of 3,4-DHPEA-EDA, whose concentration remained practically constant. Significant differences were observed in the content of 3,4-DHPEA-AC and higher values appearing in oils of the later samplings. Secoiridoid derivatives were the major fraction present in oils from the Farga cultivar (**Table 2**), and the main compound found in the first samplings was 3,4-DHPEA-EA, while in latter samplings from oils from olives with a higher RI, the most abundant compound was the 3,4-DHPEA-EDA. A new compound was identified and quantified in the secoiridoid derivatives fraction of olive oils extracted from the Farga and Morrut cultivars. This compound eluted at R_T = 27 min (peak 6′) just after 3,4-DHPEA-EDA, and it has a UV spectrum with maxima at 225 and 279.9 nm that are the same maxima as 3,4-DHPEA-EDA. The mass spectrum of that compound displayed major signals at *m*/*z* (relative intensity) 275 and 377 in the negative ESI and 433 in the positive ESI, those major signals corresponding to 3,4-DHPEA-EDA. Therefore, we can affirm that the peak 6′ is a structural isomer of 3,4-DHPEA-EDA; peak 6′ shows a lower polarity. The secoiridoid derivatives of Farga olive oils showed a significant decrease during ripening, especially 3,4-DHPEA-EA and 3,4-DHPEA-EDA isomers, while *p*-HPEA-EDA remained constant. The secoiridoid derivatives fraction was also the main phenolic fraction in Morrut olive oil (**Table 3**), and 3,4-DHPEA-EA and 3,4-DHPEA-EDA were the main compounds present. All compounds that formed the secoiridoid derivatives fraction showed the same decreasing trend throughout the ripening process although the most affected compound was the 3,4-DHPEA-EDA isomer.

Nevertheless, significant differences were observed in the phenolic content of olive drupes, mainly between hydroxytyrosol and oleuropein, both of which tended to decrease during ripening, but they were not closely related with phenolic content in olive oils and especially with hydroxytyrosol and secoiridoid derivatives (which are oleuropein and ligstroside derivatives) despite the fact that some secoiridoid derivatives showed a decreasing trend during maturation (**Tables 1**−**3**). During the extraction of olive oil, almost 80% of all oleuropein is degraded upon crushing the olive and the remaining phenolic compounds that included oleuropein are partitioned between the water and

Table 2. Effect of the Picking Date in the Phenolic Compounds of Olive Pulp, Expressed as Dry Weight, and Virgin Olive Oils from Farga Cultivar and Their Effect in the Oil Parameters[a]

peak	phenolic compd[b] (mg kg^{-1})	picking date[c] 15th Sep	1st Oct	6th Oct	20th Oct	10th Nov	30th Nov	
				olive pulp				
A	3,4-DHPEA	7170	3410	5310	3590	2570	2380	*
B	verbascoside	730	190	210	340	440	530	NS
C	oleuropein	8250	6060	2250	910	ND	ND	**
				olive oil				
1	3,4-DHPEA	0.49	0.47	0.65	0.63	0.65	0.67	**
2	*p*-HPEA	1.40	1.19	1.41	1.42	1.27	1.49	NS
3	vanillic acid	0.56	0.31	0.24	0.19	0.15	0.18	**
4	vanillin	0.53	0.58	0.35	0.44	0.23	0.24	**
5	3,4-DHPEA-AC	40	148	192	244	217	210	**
6	3,4-DHPEA-EDA	357	578	656	726	499	563	*
6′	3,4-DHPEA-EDA isomer	193	108	96	71	41	49	**
7	*p*-HPEA-EDA	39	36	46	51	37	40	*
8	lignans	139	121	133	138	128	131	NS
9	*p*-HPEA-EA	18.56	10.47	10.54	8.21	4.88	4.22	**
10	3,4-DHPEA-EA	610	612	423	367	209	208	**
11	luteolin	0.60	0.58	1.86	2.25	2.67	2.47	**
12	apigenin	0.98	0.60	0.93	1.10	0.96	0.89	NS
13	peak 13	162	129	112	120	93	52	**
14	flavonoid	1.80	1.29	1.16	1.19	0.78	0.72	**
15	peak 15	3.06	3.72	14.60	13.41	9.52	7.01	**
16	peak 16	1.36	1.12	1.76	1.79	1.20	0.86	**
17	peak 17	0.49	0.61	0.68	0.49	0.32	0.25	**
				parameter				
	total phenol (mg kg^{-1})	416	417	332	301	251	243	**
	α-tocopherol (mg kg^{-1})	190	152	172	161	141	135	*
	oxidative stability (h)	21.6	20.0	16.0	16.1	14.1	10.8	**
	K_{225}	0.303	0.360	0.371	0.300	0.267	0.245	**

[a] ND, no detected. [b] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycon. [c] Significance level. NS, not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$.

the oil phase [the partition coefficient (K_p) of hydroxytyrosol and oleuropein clearly biased to water phase (*29*)], giving rise to an important loss of phenolic compounds in vegetation waters and pomace, which could be the main cause of the low relationship between olive pulp and olive oil phenolics.

The trend of the lignans content in oils differed with olive cultivar, and this phenolic fraction was not detected in Morrut oils with the exception of the last sampling where a small amount was detected. The flavonoid fraction, formed by luteolin and apigenin and a compound that elutes at 42.3 min (peak 14), was present at very low concentrations in the oils from the three olive varieties. In the latter part of the chromatogram, formed by unknown compounds whose UV spectra are similar to those of phenolic compounds, peak 13 was the main phenolic compound, and its concentration varies in a different way in the virgin oils studied.

Tables 1−**3** show the total phenol and the α-tocopherol content, the oxidative stability, and the bitter index (K_{225}) of the Arbequina, Farga, and Morrut virgin olive oils, in relation to the RI of the fruit. The α-tocopherol content ranged from 246 mg kg^{-1} in the early samplings to 135 mg kg^{-1} in the last samplings, showing a significant decrease in all olive cultivars during September coinciding with olive drupes coloring and the beginning of black maturation **(Figure 1)**. After that significant reduction of the α-tocopherol content in olive oils during September, it remained constant during the rest of the sampling period. The three olive varieties studied followed the same decreasing trend during the black maturation process.

The stability and bitter index (K_{225}) of the oils from the three cultivars showed a very significant decreasing trend during fruit ripening (**Tables 1**−**3**). Differences in oil stability from the Arbequina cultivar (**Table 1**) between the first and the last sampling dates were not as marked as in the other cultivars studied because the total phenol content of the Arbequina oils did not show such a noticeable decrease. The high values in the oxidative stability of olive oils corresponding to early samplings could be explained by the α-tocopherol and the total phenol content of olive oils (*30*). Differences on oxidative stability of oils from different cultivars could be attributed to phenolic profile. Among secoiridoid derivatives, the main differences have been observed in 3,4-DHPEA-EDA, whose high concentration in the Arbequina olive oils gives rise to high stability, while in the Morrut cultivar the main secoiridoid derivatives were 3,4-DHPEA-EA and the 3,4-DHPEA-EDA isomer that have a proven antioxidant activity even though their activity is lower than that of 3,4-DHPEA-EDA and their content is lower than 3,4-DHPEA-EDA in the Arbequina cultivar.

The bitter index (K_{225}) evaluates the intensity of the bitter taste in virgin olive oil. This index decreases as the maturation advances, reaching values of 0.497 in the early maturation stage giving rather bitter oils, while in the latter maturation stages olive oils reach values of 0.187 as a consequence of the decrease in the olive oil phenol content and especially of a decrease on the secoiridoid derivatives, as observed by Tovar et al. (*15*).

One of the aims of this study also was to explore the organoleptic profile of the three cultivars and the changes produced in their attributes during maturation. No negative attributes were observed in this trial due to the short time spent between picking the olive drupes and the olive oil extraction. The Arbequina cultivar olive oil sensorial profile (**Figure 3**) was characterized by a high pungent and bitter attribute high intensity in the early stage of the olive maturation (September),

Table 3. Effect of the Picking Date in the Phenolic Compounds of Olive Pulp, Expressed as Dry Weight, and Virgin Olive Oils from Morrut Cultivar and Their Effect in the Oil Parameters[a]

peak	phenolic compd[b] (mg kg^{-1})	picking date[c] 15th Sep	6th Oct	3rd Nov	8th Dec	
		olive pulp				
A	3,4-DHPEA	10680	7690	4050	2480	**
B	verbascoside	180	120	140	190	**
C	oleuropein	3990	9200	3900	820	*
		olive oil				
1	3,4-DHPEA	0.53	0.49	0.59	0.66	NS
2	*p*-HPEA	1.07	0.79	0.65	0.59	**
3	vanillic acid	0.75	0.77	0.06	0.16	**
4	vanillin	1.60	1.92	1.22	0.73	**
5	3,4-DHPEA-AC	29	70	87	65	NS
6	3,4-DHPEA-EDA	771	796	248	280	**
6′	3,4-DHPEA-EDA isomer	234	95	31	75	**
7	*p*-HPEA-EDA	120	115	38	30	**
8	lignans	0	0	0	15	NS
9	*p*-HPEA-EA	17.58	10.25	3.57	5.76	**
10	3,4-DHPEA-EA	937	609	225	359	**
11	luteolin	0.20	0.16	ND	ND	**
12	apigenin	0.41	1.47	ND	ND	**
13	peak 13	119	116	195	151	**
14	flavonoid	0.89	0.82	ND	ND	**
15	peak 15	1.04	0.82	ND	ND	**
16	peak 16	0.62	2.93	ND	ND	**
17	peak 17	0.55	0.57	ND	ND	**
		parameter				
	total phenol (mg kg^{-1})	475	374	139	201	**
	α-tocopherol (mg kg^{-1})	246	224	175	154	*
	oxidative stability (h)	17.5	16.0	8.4	8.8	**
	K_{225}	0.422	0.421	0.187	0.221	**

[a] ND, no detected. [b] 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycon. [c] Significance level. NS, not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$.

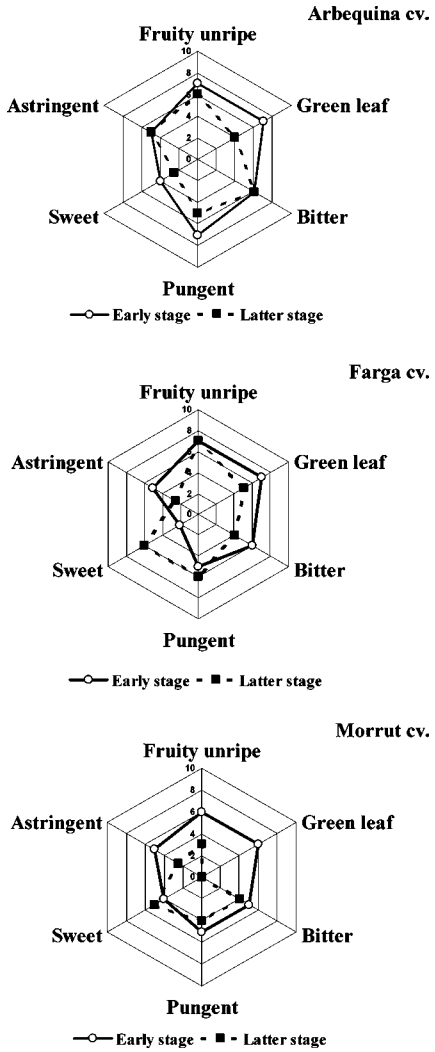

Figure 3. Effect of the olive maturation stage in the sensorial profile of the olive oils from Arbequina, Farga, and Morrut cultivars. Values are the means of four independent values.

while in the later stages (November) significant decreases were observed in the sweet, pungent, and unripe attributes. The Farga cultivar sensorial profile was characterized by less sweet and pungent attributes in unripe oils (early stage, September), whereas in riper olive oils the sweet attribute increased in intensity very significantly while bitter and green leaf attributes suffered a decrease in intensity as a consequence of the ripening process. Finally, the Morrut cultivar olive oil sensorial profile showed more balanced oils, having positive attributes between 5 and 6 in unripe oils (early stage), whereas in riper oils (latter stage) the green leaf attribute disappeared and lower values of the fruity unripe, astringent, pungent, and bitter attributes were observed. Moreover, other attributes were observed in olive oils such as artichoke, unripe almond, and anise-fennel. Lower intensities of the green almond attribute were detected in samples proceeding from riper olives (latter stage). The artichoke attribute was more intense in the Morrut cultivar, while the anise-fennel attribute had higher intensities in the Arbequina cultivar.

To sum up, the olive oil yield followed a rising trend as a consequence of the loss of moisture during black maturation that facilitates the oil extraction. Consequently, in the last stages of maturation, yields are obtained but the olive oil presents lower levels of antioxidants such as α-tocoferol and phenol content. Significant differences have been shown in the phenolic content of olive drupes, mainly between hydroxytyrosol and oleuropein, both of which tended to decrease during ripening while the Morrut variety could be easily identified on first samplings by its higher amounts of verbascoside. On the other hand, a general decreasing trend was observed in the phenolic content of olive oils over the wide range of phenolic compounds present with the exception of 3,4-DHPEA-AC, which, in the three varieties, was being accumulated during the ripening process. This was probably a result of a degradation of secoiridoids derivatives. Differences in the HPLC profile between varieties were observed that included the absence of or presence of very low amounts of lignans in olive oils proceeding from the Morrut cultivar, and the presence of three peaks after elution of 3,4-DHPEA-EDA, which had the same UV and MS spectra but eluted in different R_T in the Farga and Morrut cultivars, could be used as differentiating parameters. These differences between oils jointly with verbascoside in olive drupes could be used as parameters to differentiate between olive varieties.

Finally, no negative attributes were observed in the sensory analysis of the oils. Sensory profile differences were observed between olive cultivars as well as differences due to the ripening process such as the presence of astringent, pungent, and bitter attributes in lower intensities and the increase of the sweet attribute in olive oils proceeding from the Farga and Morrut cultivars.

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