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An explanation for the natural de-bittering of Hurma olives during ripening on the tree

E. Susamci[a], C. Romero[b], O. Tuncay[c] and M. Brenes[b,✉]

[a]Olive Research Institute, Üniversite Caddesi No: 43 35100 Bornova. Izmir, Turkey
[b]Instituto de la Grasa (IG-CSIC), Campus University Pablo de Olavide, Ctra. Utrera km 1, 41013-Seville, Spain
[c]Faculty of Agriculture, Ege University, 35100 Izmir, Turkey

✉Corresponding author: brenes@cica.es

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SUMMARY: Harvested olives require further processing to make them edible due to their content in the bitter substance oleuropein. However, some olives of the Erkence cultivar naturally de-bitter on the tree giving rise to the so-called Hurma olives. In this study, the evolution of the chemical characteristics of Erkence and Hurma olives harvested from the northeast and southwest area of trees located in the Karaburun Peninsula was assayed. It was confirmed that the oleuropein content in Hurma olives was much lower (< 2000 mg/kg fresh weight) than Erkence, which reached 35.000 mg/kg fresh weight at the beginning of the season. In addition, no free or polymerized anthocyanins were found in Hurma fruit in contrast to ripened Erkence fruit. The concentration of glucose was also lower in Hurma than Erkence olives. These results suggest that the enzymatic oxidation of oleuropein could be responsible for the natural de-bittering of Hurma olives during their ripening on the tree.

KEYWORDS: *Anthocyanin; Bitterness; Olive; Phenolic compounds; Sugar*

RESUMEN: ***Una explicación para el desamargado natural de aceitunas Hurma durante su maduración en el árbol.*** Las aceitunas recién cogidas del árbol necesitan ser procesadas para hacerlas comestibles, debido a su contenido en el compuesto amargo oleuropeína. Sin embargo, algunas aceitunas de la variedad Erkence desamargan de forma natural en el árbol dando lugar a las aceitunas conocidas como Hurma. En este trabajo se han analizado las características químicas de aceitunas Erkence y Hurma recolectadas de la zona noreste y suroeste de árboles situados en la provincia de Karaburun. Se ha confirmado que el contenido en oleuropeína de aceitunas Hurma es muy inferior (< 2000 mg/kg) que Erkence, las cuales alcanzaron una concentración en dicha sustancia hasta de 35.000 mg/kg al principio del periodo de maduración. Además, no se encontraron en aceitunas Hurma antocianinas ni libres ni polimerizadas, a diferencia de Erkence. Estos resultados indican que la oxidación enzimática de la oleuropeína podría ser la responsable de la eliminación del amargor de forma natural en aceitunas Hurma durante su maduración en el árbol.

PALABRAS CLAVE*: Aceituna; Amargor; Antocianina; Azúcar; Compuestos fenólicos*

ORCID ID: Susamci E http://orcid.org/0000-0003-1956-4634, Romero C http://orcid.org/0000-0003-1885-1770, Tuncay O http://orcid.org/0000-0002-5218-1056, Brenes M http://orcid.org/0000-0001-7419-4061

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1. INTRODUCTION

Olives are generally produced in Mediterranean countries, where there are about 8 million hectares of cultivated olives trees. Olive oil and table olives are the two food products obtained from these fruits, which are key components of the Mediterranean diet. Olives cannot be consumed immediately after harvest because of their high phenolic content, especially the bitter glucoside oleuropein (Ramírez *et al*., 2014). To remove the bitter taste, some processing methods have been developed such as Spanish-style green olives and California black oxidized olives which consist of removing the bitterness by treating the fruit with a dilute solution of NaOH. Other trade preparations such as green and black natural olives involve the direct brining of the fruit without any alkaline treatment, and the bitterness disappears slowly over time (Ramírez *et al*., 2015).

There is an olive cultivar in Turkey, called Erkence, which can become edible naturally while still on the tree, when it is grown in the Karaburun Peninsula. This "natural de-bittering" gives rise to olives called Hurma (Susamci, 2011). From October to December, the color of the skin and the flesh of the fruits become dark-brown, they lose some water and fall to the ground. The fruits of other varieties grown in the same region cannot de-bitter on the tree. Although Erkence fruits have the ability to de-bitter on the tree, not all of them undergo this process. Hurma olives that fall to the ground are continuously collected by growers, and they can be directly consumed without any post-harvest processing. Therefore, these olives are a healthy food product suitable for people seeking salt-free table olives.

There are only a few studies on Hurma olives, whose de-bittering on the tree has been associated to changes in phenolic compounds during the late period of maturation (Aktas *et al*., 2014a). These olives possess a higher linoleic acid content than Erkence, which might be an indication of increased desaturase enzyme activity during the natural de-bittering phase (Aktas *et al*., 2014b). In addition, it has been suggested that the fungus named *Phoma oleae* could be involved in the hydrolysis of oleuropein (Panagou, 2006), although it is not the prevalent microorganism on the surface of Hurma olives (Sozvilen and Baysal, 2016). There are many sweet olive varieties such as Dhokar, Gordal/Sevillano and Trouba Thassos but all of them need post-harvest processing to make them palatable (Panagou, 2006; Menz and Vriesekoop, 2010; Rigane *et al*., 2011). In contrast, Hurma olives are currently consumed after harvesting without any brining process.

The phenomenon of Hurma olive formation on the tree of the Erkence fruit is still unknown. According to growers, climate is a very important factor for the development of this process. They claim that natural de-bittering needs northern winds and dew. In this study, the effects of fruit location on the tree and the altitude and distance from the sea of the orchards, on some chemical compounds in Hurma and Erkence olives grown in the Karaburun Peninsula were investigated. An explanation for the natural de-bittering of the Hurma olives as well as their color changes during formation has been proposed.

2. MATERIALS AND METHODS

2.1. Olive samples

Two orchards located in the Karaburum Peninsula (Turkey) with olive trees of the Erkence cultivar were chosen for the experiments due to their availability for Hurma olive formation. Orchard A was cultivated at sea level (100 m distance from the sea, latitude: 38º 32′ 56′′ N, longitude: 26º 34′ 56′′ E) and orchard B at 174 m above the sea (1.4 km distance from the sea, latitude: 38º 33′ 42′′ N, longitude: 26º 33′ 5′′E). Both Hurma (naturally de-bittered Erkence) and Erkence (not naturally de-bittered) olives were collected manually during the maturation period from the northeast (facing the sea) and southwest (inland) sides of triplicate trees. Olive samples were picked for eleven weeks in the first season (2014/15) and nine weeks in the second season (2015/16), starting from the end of September. It must be noted that both Hurma and Erkence olives were taken from the same trees throughout the maturation period. Fruit samples were transferred to the laboratory in PE bags, and the maturity index and moisture were determined. For other analyses, olives were frozen at -20 ºC and freeze dried, after the removal of the stones.

2.2. Maturity index

One hundred olives were selected at random, classified into seven groups according to their color (green, black, reddish brown, etc.) and the number of fruits in each group was determined. Black fruits were cut to examine the percentage of black or purple coloring in the flesh. The number of fruits in each class was multiplied by the coefficient of the class, and the maturity index was calculated according to the formula given by Morello *et al*., (2005).

2.3. Analysis of moisture

The water content was measured on fresh olive paste by drying in an oven at 105 ºC until constant weight was obtained.

2.4. Analysis of phenolic compounds

Around 1.5 g of freeze dried olive pulp was mixed in an Ultra-turrax homogenizer with 30 mL of dimethylsulfoxide (DMSO). After 30 min of resting contact,

the mixture was centrifuged at 6000g, and 0.25 mL of the supernatant were diluted with 0.5 mL of DMSO plus 0.25 mL of 0.2 mM syringic acid in DMSO (internal standard). The mixture was filtered through a 0.22μm pore size nylon filter, and an aliquot (20 μL) was injected into the chromatograph. The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600E pump and a Waters 996 diode array detector (Waters Inc. Milford, MA, USA). A Spherisorb ODS-2 (5 μm, 25 cm x 4.6 mm i.d., Waters Inc.) column was used. Separation was achieved using an elution gradient with an initial composition of 90% water (pH adjusted to 2.7 with phosphoric acid) and 10% methanol. The concentration of the latter solvent was increased to 60, 70 and 100% in 5-min periods. A flow rate of 1 mL/min and a temperature of 35 ºC were used. Chromatograms were recorded at 280 nm for phenolic compounds (Ramírez *et al*., 2014).

The identification of the phenolic compounds in the extract was made using a HPLC-MS system that consisted of a Waters 2695 Alliance with a pump, column heater and autosampler modules, and the detection was carried out with a Waters 2998 photodiode array detector and a mass single-quadrupole detector (QDa, Waters, USA). The QDa mass detector was operated in the negative mode (ESI-), the capillary voltage was set at 0.8 kV, the cone voltage to 15 V, and nitrogen was used as nebulizer gas with de-solvation temperature set at 600 ºC. The flow rate was 1 mL/min, and the column, solvent and gradient conditions were the same as mentioned above.

2.5. Total pigment analysis

After dissolving 0.1 g of freeze-dried fruit sample in 1.5 mL of 1 M HCl, the mixture was first vortexed for 1 min., sonicated for 1 min, vortexed again for 1 min., and then centrifuged. The supernatant was then filtered through a 0.22 μm pore size filter, and diluted 10 fold with 1 M HCl. The absorbance spectrum of the extracts (λ_{400}–λ_{700}) was measured using a Shimadzu UV-vis spectrophotometer, which was equipped with a computer software program to calculate the CIELAB parameters (Romero and Bakker, 2000).

2.6. Analysis of free anthocyanins

Anthocyanins were extracted from a 0.1 g sample of freeze-dried olive sample with a solution of methanol:hydrochloric acid (99:1, v/v, 0°C) as described elsewhere (Romero *et al*., 2002). Briefly, the mixture was centrifuged at 9000 g for 5 min (10 ºC), and the extraction was repeated 6 times. The methanolic extracts were concentrated under vacuum at 30 ºC to water residue and made up to 25 mL volume with acidified dionized water. A washing step with hexane was required to remove fat from the extract, and the residual solvent was eliminated by purging nitrogen. An aliquot of 20 μL was injected into the chromatograph. The HPLC system consisted of a Waters 2695 Alliance with a pump, column heater (40 ºC) and autosampler modules, and the detection was carried out with a Waters 2998 photodiode array detector (Waters Inc.). A 25 cm × 4.6 mm i.d., 5-μm Extrasil ODS-2 (Technokroma, Barcelona, Spain) column was used and the elution conditions were as follows: flow rate = 1 ml/min; solvent A, water with 1% perchloric acid, solvent B, methanol. The mobile phase consisted initially of 80% of A; using a linear gradient, the concentration of methanol was increased to 50% over 35 min, to 98% at 40 min, held for 2 min at 98% of B to wash the column, and then returned to the initial conditions (20% of B) for 10 min. Chromatograms were recorded at 520 nm.

2.7. Analysis of sugars

Freeze-dried fruit (1 g) was mixed with 20 mL of boiling water and vortexed for 1 min, kept in an ultrasonic bath for 3 min, vortexed again for 1 min, and the mixture was centrifuged at 9000 g for 5 min. The mixture was filtered through filter paper using a vacuum, and another 20 mL of hot water were added and filtered again. The filtrate was then transferred to a 50 mL volumetric flask containing 2 mL of sorbitol as internal standard (7.5%, w/v) and made up to volume. The solution was kept at 5 °C for 24 h to remove lipids and subsequently filtered through a 0.22 μm pore size nylon filter. Two milliliters of the clarified liquid were put into contact with 1 g of the acidic resin Amberlite IR-120 and 1 g of the basic resin Amberlite IRA-93. Samples were shaken occasionally for 30 min, and 1 mL of the solution was centrifuged at 9000 g for 3 min. An aliquot of 20 μL was injected into the chromatograph. The HPLC system consisted of a Waters 2695 Alliance with a pump and autosampler included; the detection was performed with a Waters 410 refractive index detector. A Rezex RCM-Monosaccharide Ca+ (8%) column (300 × 7.8 mm i.d., Phenomenex) held at 85 °C and deionized water as eluent at 0.6 mL/min were used (Medina *et al*., 2007).

2.8. Statistical analysis

Statistical comparisons of the mean values for each experiment were performed by one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test ($p < 0.05$) using Statistica software version 8.0 (Stat-Soft, 2001).

3. RESULTS AND DISCUSSION

The qualitative HPLC-MS analysis of olive extracts allowed for the identification of 11 phenolic compounds in Erkence and Hurma fruit, and the

quantification of these substances by HPLC-DAD confirmed oleuropein as the main compound in olives throughout their ripening period (Ramírez *et al*., 2014; Talhaoui *et al*., 2015). Figure 1 shows the content of oleuropein in Erkence fruit during the ripening phase on olive trees cultivated in two different locations for two seasons. First, it must be noted that previous studies reported a very much lower concentration of oleuropein in Erkence olives that the content which is depicted in Figure 1 (Arslan, 2012; Aktas *et al*., 2014a). In fact, the latter researchers found a higher concentration in hydroxtyrosol and apigenin than oleuropein in these olives; whereas we did not detect the presence of these substances by using HPLC-MS.

The concentration of oleuropein decreased significantly during ripening in Erkence olives during the two seasons, which was more pronounced from the first to the 5th–7th week, which is a pattern previously described for other olive varieties (Menz and Vriesekoop, 2010; Talhaoui *et al*., 2015) but not in Erkence fruit (Aktas *et al*., 2014a).

Regarding the orchard location, both orchards are cultivated in the Karaburun Peninsula (Izmir, Turkey) under common Mediterranean climate characteristics with drought conditions, and orchard A lying near the sea and B in the mountains. The concentration of oleuropein was higher in the olives harvested from orchard B than A during the first week of the 2014/2015 season (Figure 1) but the opposite trend was observed during the 2015/2016 season, and no significant differences were found for mature olives (weeks 5-11). The effect of cultivation environment on the phenolic composition of fruits and leaves has been widely reported but many contradictory results have been found. Olive oils from fruit grown at high altitude had a higher content in phenolic compounds than those at a low altitude (Di Vaio *et al*., 2013), and vice-versa (Mousa and Gerasopoulos, 1996). Contradictory results have also been reported for the accumulation of anthocyanins in berries (Guerrero-Chavez *et al*., 2015; Zoratti *et al*., 2015). Phenolic compounds contribute to the protection of plants against UV radiation, and leaves and fruit cultivated at high altitude are currently richer in these substances. However, many other variables affect the content of phenolic compounds in plants such as soil, climate, and agronomic conditions.

In this study, samples of Erkence olives were collected from the northeast and southwest orientation of trees cultivated in both orchards A and B. No effect on the oleuropein composition due to tree orientation was found (Figure 1). Gómez-del-Campo and García (2012) obtained a higher phenolic concentration in olive oils extracted from fruit located at higher layers of the olive trees than lower layers, and Romero *et al*., (2016) reported that olive oils from sunny areas had a higher concentration

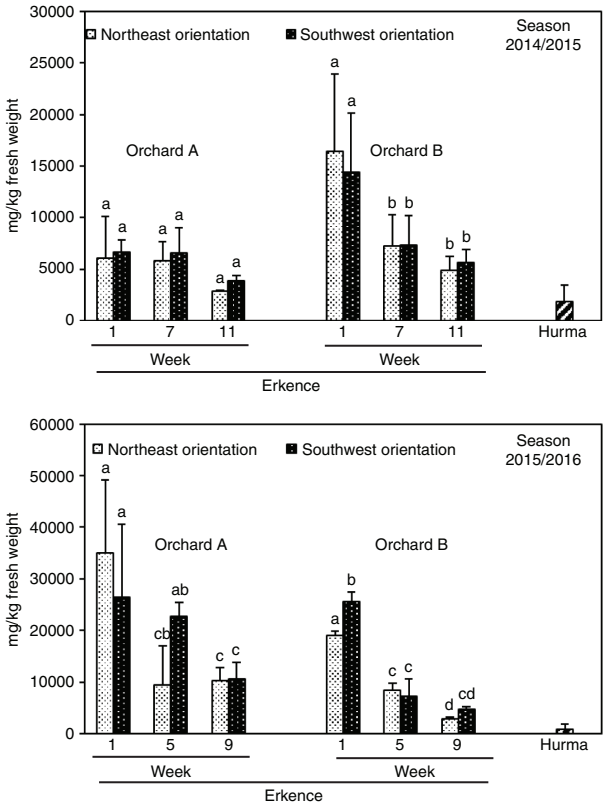

FIGURE 1. Effect of olive tree orientation and ripening on the concentration (mg/kg fresh weigh) of oleuropein in Erkence and Hurma olives. Three samples of Erkence olives were analyzed for each sampling time. Eight samples of Hurma olives were collected from orchards A and B at week 7 during the season 2014/2015, and 11 samples at weeks 5 and 9 during the season 2015/2016. Vertical bars with different letters indicate significant differences according to Duncan's multiple-range test ($p < 0.05$).

of these substances than those from shady areas. A trend was observed for the rest of the phenolic compounds analyzed in Erkence olives (Table 1), although it was not statistically significant. It must be noted that the synthesis of phenolic compounds is influenced by solar radiation, the longer the hours of exposure to solar radiation of fruit and leaves, the higher the concentration of certain phenolic compounds will be found in these materials (Spayd *et al*., 2002; Morales *et al*., 2010).

Similar to oleuropein, hydroxytyrosol 1-glucoside, oleuropein aglycon, ligustroside, rutin, comselogoside and others decreased their concentrations in Erkence olives with ripening. In contrast, the hydroxytyrosol 4-glucoside content increased with olive maturation as has been previously reported for other olive varieties (Romero *et al*., 2002). The concentration of all these compounds was also higher in olives harvested during the second than the first season (Table 1), which also occurred with the content in oleuropein (Figure 1).

TABLE 1. Influence of harvesting season and olive tree orientation on the concentration of phenolic compounds (mg/kg fresh weight) other than oleuropein during ripening of Erkence and Hurma olives from orchard B

	Oleuropein aglycon	Hydroxytyrosol 1-glucoside	Hydroxytyrosol 4-glucoside	Ligustroside	Rutin	Comselogoside	Others[a]
***(2014/2015)* Erkence**							
Week 1 (N)[d]	3315a	110a	408a	777a	568a	189a	69a
Week 1 (S)	4421a	108a	285a	754a	779a	201a	109a
Week 7 (N)	1043b	25b	585a	334b	324b	101b	48a
Week 7 (S)	1504b	23b	492a	383b	313b	123b	42a
Week 11 (N)	1000b	24b	466a	232b	331b	68b	113a
Week 11 (S)	949b	35b	638a	251b	324b	80b	94a
Hurma (week 7)	65 ± 144[b]	16 ± 26	191 ± 104	304 ± 239	161 ± 141	37 ± 38	11 ± 11
***(2015/2016)* Erkence**							
Week 1 (N)	2044a	359a	572a	1174a	289a	123a	113a
Week 1 (S)	2662a	489b	739a	1365a	567b	176a	194ab
Week 5 (N)	4030a	259ac	658a	546b	202a	118b	44ac
Week 5 (S)	5168b	220acd	843a	518b	306a	117b	70ab
Week 9 (N)	2395a	99d	613a	180c	171a	71b	49ac
Week 9 (S)	2440a	150d	1344a	249c	176a	70b	98ab
Hurma (weeks 5 and 9)	123 ± 286[c]	13 ± 18	247 ± 156	202 ± 143	36 ± 45	19 ± 17	6 ± 18

[a] Others is the sum of verbascoside, caffeoyl ester of secologanoside, caffeic and *p*-coumaric acids.
[b] Mean of 5 samples ± standard deviation.
[c] Mean of 11 samples ± standard deviation.
[d] N, northeast; S, southwest. Column values for each season followed by the same letter did not differ at the 5% level according to Duncan's multiple-range test.

Some of the Erkence olives shrivel and lose moisture and bitterness during their ripening on the tree, giving rise to the well-appreciated Hurma olives. The data depicted in Figure 1 confirm the low concentration of oleuropein in Hurma olives in comparison with Erkence fruit for the two seasons analyzed. The contents of oleuropein aglycon, hydroxytyrosol glucosides and the rest of the phenolic compounds were also much lower in Hurma than Erkence olives (Table 1). A previous study reported a low concentration of oleuropein in Hurma olives but also a very high content in hydroxytyrosol and apigenin (Aktas *et al*., 2014a), whose presence in Hurma olives has not been confirmed in this work by using HPLC-MS.

To our knowledge, there is not a clear explanation for the natural de-bittering of Hurma olives, although it has been suggested that the infection of the fruit by *Phoma olea* could be a possibility (Atkas *et al*., 2014b). One of the phenomena which occurred during the transformation of Erkence to Hurma olives is a decrease in moisture in the fruit. This parameter ranged in Hurma olives between 30–40% (Figure 2) whereas Erkence had around 50%. *Phoma* fungus causes irregular necrotic patches in fruits, a drop in phenolic compounds and an increase in peroxidase and polyphenoloxidase activity (Bugbee, 1975; Hura *et al*., 2014). Also, an anthracnose disease originated by *Colletottrichum acutatum* gives rise to desiccated fruits (Moral *et al*., 2014) but

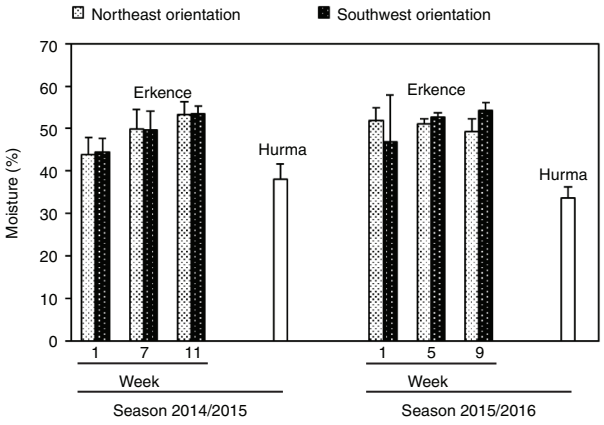

FIGURE 2. Effect of olive tree orientation and harvesting season on the moisture (%) of Erkence and Hurma olives. Fruit were collected from orchard B. Five samples of Hurma olives were harvested at week 7 during the first season, and 11 samples of these olives were harvested at weeks 5 and 9 during the second season. Standard error is shown on the bars.

Hurma olives look like dry-salted olives rather than mummified fruit. Frost damage during the harvest period also produces olive shriveling, softening and water loss, and it could be another explanation for the formation of Hurma olives but it must be confirmed. In fact, frost causes cell death and dehydration in fruits and a high oxidation of cell contents

may occur as a result of contact between oxidase enzymes and phenolic substrates, which has been reported for the decrease in the bitter taste in olive oils affected by freeze injuries (Morello *et al*., 2003).

Erkence olives change their color during ripening in a similar way to many other olive varieties. The surface color turns to yellow, purple and finally black as a consequence of anthocyanin synthesis that also continues inside the pulp. In contrast, Hurma olives change their color directly from green-yellow to brown or dark brown not only on their surface but also in their pulp. An explanation for these color changes in Hurma olives remains unsolved. Erkence olives with a maturation index of 3-4 had a significant content in anthocyanins (Table 2). This maturation index means olives with purple or black surface color and white color inside the pulp so that it was predictable to find anthocyanins in these olives, mainly cyanidin-3-rutinoside and cyanidin-3-glucoside (Romero *et al*., 2002). Surprisingly, free anthocyanins were not detected in Hurma olives which were classified with a maturation index of 7. In addition, the color parameters (*L**, *a**, *b**, λ_{520}) of olive extracts from Erkence and Hurma olives revealed that those from Erkence had a purple/red color whereas those of Hurma had a slightly yellow color (Table 2). Anthocyanins are found free in raw olives and can be oxidized and polymerized during processing (Romero and others 2004). The results presented in Table 2 indicate the absence of these substances either free or polymerized in Hurma olives so that their dark brown color could be formed from the oxidation of *o*-diphenols, particularly oleuropein. The oxidation of the latter substance gives rise to non-bitter substances (García *et al*., 2008), and it has been proposed as the explanation for the de-bittering of black dry-salted and dried green olives (Ramírez *et al*., 2013; Piscopo *et al*., 2014).

Another difference found between Erkence and Hurma olives was their content in sugars (Table 3). As expected, glucose, fructose and mannitol were

TABLE 2. Color parameters (L*, a*, b*, λ_{520}) of an acidic extract of Erkence and Hurma olives, and concentration of anthocyanins (mg/kg fresh weight) in the pulp of

Olive type	Maturity index	Color parameters of extract				Anthocyanins	
		L*	a*	b*	λ_{520}	Cyanidin-3-glucoside	Cyanidin-3-rutinoside
Erkence	3.8	72.7	51.4	13.0	>2	341 ± 145[a]	1703 ± 597
Erkence	3.2	83.9	28.2	3.6	0.42	173 ± 6	1030 ± 97
Erkence	3.6	73.2	50.6	14.6	1.32	46 ± 4	292 ± 13
Hurma	7	96.3	0.2	5.4	0.04	nd[b]	nd
Hurma	7	96.7	-0.4	8.2	0.05	nd	nd
Hurma	7	97.0	-0.5	6.3	0.05	nd	nd

[a]Standar deviation.
[b]nd, not detected.

TABLE 3. Concentration of main sugars (mg/kg fresh weight) in the pulp of Erkence and Hurma olives collected from orchard B

	Olive type	Tree orientation	Week of harvesting	Glucose	Fructose	Mannitol
Season 2014/2015						
	Erkence	Northeast	1	12901a[a]	4435a	9380a
	Erkence	Southwest	1	14295a	4350a	9760ab
	Erkence	Northeast	11	8971a	6304a	4955ab
	Erkence	Southwest	11	10297a	7106a	5193abc
	Hurma	Northeast	11	2323b	4128a	8570a
	Hurma	Southwest	11	2539b	3256a	8255a
Season 2015/2016						
	Erkence	Northeast	1	8690a	2333a	2942a
	Erkence	Southwest	1	9969a	3527a	4004a
	Erkence	Northeast	8	9442a	2557a	4866a
	Erkence	Southwest	8	9187a	2383a	2776a
	Hurma	Northeast	9	1682b	2672a	5056a
	Hurma	Southwest	9	1928b	2145a	4083a

[a] Data are the mean of triplicates (Erkence olives) or duplicates (Hurma olives). Column values for each season followed by the same letter did not differ at the 5% level of significance according to the Duncan's multiple-range test.

the main free sugars detected in the olives (Marsilio *et al*., 2001), and a general decrease from week 1 to weeks 8-11 was observed for Erkence olives in agreement with previous reports (Menz and Vriesekoop, 2010). A trend to higher sugar content in Erkence olives harvested from the southern area of the trees than the north was also detected although it was not always significant. Surprisingly, the content of glucose was much lower in Hurma than Erkence olives but this effect was not detected for the concentration of fructose and mannitol, and it was not observed in a previous study (Aktas *et al*., 2014b). It is well-known that respiration is enhanced in freeze-injured and fungus infected fruits (Fuchs *et al*., 1975; Liu *et al*., 2005), and olives consume mannitol and fructose at a higher rate than glucose during their post-harvest period (García *et al*., 1995). Hence, Hurma olives de-bitter during their ripening on the tree and they also consume a high content of glucose during this period but not mannitol or fructose.

4. CONCLUSIONS

This study confirmed the low concentration of phenolic compounds in Hurma olives, particularly the bitter glucoside oleuropein, in comparison with that of Erkence fruit. Moreover, dark-brown Hurma olives did not contain free or polymerized anthocyanins as opposed to black Erkence which possessed free cyanidin-3-rutinoside and cyaniding-3-glucoside. These pigments therefore are not involved in the dark color of Hurma olives but polymers formed during the enzymatic oxidation of oleuropein must be responsible for this dark-brown color. Therefore, Hurma olives lose their bitterness during maturation on the tree due to the enzymatic oxidation of oleuropein, which gives rise to non-bitter substances, as it has also been demonstrated for the de-bittering of black dry-salted and dried green olives (Ramírez *et al*., 2013; Piscopo *et al*., 2014).

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