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α-Tocopherol and fatty acids contents of some Tunisian table olives (*Olea europea* L.): Changes in their composition during ripening and processing

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Abstract

An experimental investigation was carried out on Tunisian olive-fruits of *Meski*, *Sayali* and *Picholine* cultivars. α-Tocopherol and fatty acids (FA) contents were analyzed, during both ripening and processing, according to the Spanish style. The relationship between oil, unsaponifiable and α-tocopherol contents was determined only during ripening. A genetic effect on FA composition was observed throughout the sampling periods. The highest oleic acid content was found in *Sayali* cultivar at green stage (78.5% of total FA). α-Tocopherol was positively correlated with unsaturated FA content ($R = 0.71$, $p < 0.05$), and oil amount ($R = 0.984$; $R = 0.976$; $R = 0.952$, $p < 0.05$ for *Picholine*, *Sayali* and *Meski*, respectively), but it was not correlated with unsaponifiable matter. In processed olive-fruits, the results showed primarily, that processing according to the Spanish style is not restricted to green olive-fruits but can be successfully used in cherry olives with guaranteed quality and nutritional value of processed product (*Meski* and *Picholine*) related to FA content. Secondly, both α-tocopherol and FA amounts decreased during processing for all cultivars. This decrease was cultivar dependent. It was more pronounced in the black fruit than in the green one for the same cultivar. During fermentation, pH variation showed the same profile in all cultivars. Final pH values at the end of fermentation depend on the concentration of free FA (acidity) in the brine.

Keywords: Table olive; α-Tocopherol; Fatty acids; Ripening; Anaerobic processes; Cherry olives

1. Introduction

Olea europea L. is the most widespread and important plant in the Mediterranean countries. Indeed, 98% of olive production worldwide is concentrated in the Mediterranean basin. A considerable part of olive production is processed. According to statistical data (IOOC, 2003), Tunisian table olive annual production is about 23,000 tons.

Table olive is a very important fermented food of the Mediterranean countries. Olive-fruit is highly appreciated for its good taste, as well as for its nutritional properties. The nutritional benefits are mainly related to α-tocopherol and FA contents (Ribarova, Zanev, Shishkov, & Rizov, 2003). In fact, UFA participate in the regulation of cholesterol level (Delplanque, 1998). Monounsaturated fatty acid stimulates transcription of the RNAm of LDL-cholesterol receptor (Sorci, Wilson, Johnson, & Rudeell, 1989) and reduces breast cancer risks. Moreover, α-tocopherol defends the body against free radical attacks by protecting polyunsaturated fatty acids (Cheeseeman & Slater, 1993; Doelman, 1989; Kamal-Eldin & Andersson, 1997) and

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preventing the body from cancer and arteriosclerosis (Armstrong, Paganga, Brunev, Miller, & Nanchahal, 1997; Caruso et al., 1997; Nicolaiew et al., 1998).

Table olive differs from other fermented foods (carrots, cabbage, pumpkins and beans) in its chemical composition due to its relatively low sugar level (2–5%), high fat content (20–35%), and its bitter taste caused by oleuropein (Fleming, Walter, & Etchells, 1973). Because of those characteristics, the olive-fruits are not edible without prior treatment.

The processing of green olives includes initial treatment with lye, which hydrolyses the bitter glycoside oleuropein, molecule responsible for the bitter taste, (Brenes & de Castro, 1998). This treatment affects olive skin (Marsilio, Lanza, & De Angelis, 1996) by increasing the permeability of cellular membrane and moving some biochemical compounds into the surrounding liquid (Garrido Fernández, Fernández Diez, & Adams, 1997) such as α-tocopherol and free FA. Then, after washing olives with water, to eliminate the excess of alkali, fruits are stored in brine (5–10% NaCl) from two to six months, depending on the needs of production. The developed acidity and pH drop are the determining factor for the success of fermentation (Spyropoulou, Chorianopoulos, Skandamis, & Nychas, 2001).

Many methods for determining tocopherols composition have been published using normal phase HPLC (Montedoro, Servili, Baldioli, & Miniati, 1992; Pirisi et al., 1997) or reversed-phase HPLC (Abidi & Mounts, 1997; Dionisi, Prodolliet, & Tagliaferri, 1995; Richheimer, Kent, & Bernart, 1994). Most procedures require lengthy sample preparation (saponification and extraction) for the analysis of α-tocopherol (Katsanidis & Addis, 1999). In contrast, in other research α-tocopherol was determined directly from the oil (Kochar & Rossell, 1990).

Although α-tocopherol and FA compositions of olive oil have been studied extensively (Maria & Out, 2003; Salvador, Aranda, & Fregapane, 2001; Shela et al., 2003), information on the effects of anaerobic process and ripening on α-tocopherol and FA contents of table olive are scare. In the present study, the α-tocopherol and FA contents were determined in each stage (green, cherry and black occur from the 33rd to the 38th week after flowering date) of fresh olives and were compared to their contents of the same processed fruits. Processing according to the Spanish style is used normally to process green olive-fruits. Thus, in our research we confirmed that Spanish style processing can be successfully used in cherry olives with guaranteed quality of processed product.

2. Material and methods

2.1. Reagents and standard

Isopropanol (2-propanol) and *n*-hexane 95%, solvents of HPLC grade, were purchased from Panreac Quimica SA. (Barcelona, Spain). Chloroform and petroleum ether were from Fisher Scientific SA (Loughborough, Spain). Ethanol was purchased from Scientific Limited (Northampton, UK). The standard dl-tocopherol was from CN Biosciences Inc. (La Jolla, CA).

2.2. Samples

Olive-fruits of *Meski*, *Sayali* and *Picholine* cultivars were hand-harvested from the north of Tunisia from the 33rd to the 38th week after flowering (WAF) date. Green, cherry and black stages occur from the 33rd to the 34th WAF, from the 35th to the 36th WAF and from the 37th to the 38th WAF, respectively. Olive-fruits, from each stage, were processed separately during four months according to the anaerobic method. Only healthy fruits, without any kind of infection or physical damage, were processed. Chemical and physical characteristics of these cultivars were determined both in fresh and proceed olives.

2.3. Processing of olive-fruits

The processing was carried out in our laboratory according to the anaerobic method (IOOC, 2003). The olives were treated by sodium hydroxide solution (0.5 M NaOH), then washed with tap water for 12 h and finally placed in sterilized glass bottles containing sterile brine. The concentration of sodium hydroxide was kept constant throughout the fermentation. This solution must be adjusted 24 h in advance (5–10% NaCl and pH 6.4) in order to avoid the formation of bubbles on the exocarp of fruits caused by the elevated temperature of the alkali solution. The brine level was adjusted, when necessary, with fresh sterile brine to ovoid air penetration (to avoid growth of oxidative yeasts and moulds on the surface).

2.4. Extraction procedures

Extraction of oil was carried out from the dry weight of olives (Olives were dried at 20 °C in dry air sterilizers) with petroleum ether in a Soxhlet apparatus for 4 h at 42 °C. The solvent was removed by rotary evaporator. Oil was weighed and stored at −10 °C. The oil content was determined as the difference in weight of dried olive sample before and after the extraction (AOCS, 1989).

Unsaponifable matter was extracted by saponifying 5 g of lipid extracts with 50 ml ethanolic KOH 12% (w/v) and heating at 60 °C for 1.30 h. After cooling, 50 ml of H_2O was added and the unsaponifiable matter was extracted four times with 50 ml of petroleum ether. The combined ether extract was washed with 50 ml EtOH–H_2O (1:1; v/v). The extracted ether was dried over anhydrous Na_2SO_4 and evaporated to dryness using N_2. The dry residues were dissolved in chloroform for further analysis.

2.5. Analytical methods

α-Tocopherol content was determined according to AOCS Method (1989). An amount of 0.2 g of extracted

oil was dissolved in 3 ml of hexane, and then 20 μl of the solution was hand-injected into the HPLC (HP 1100, Agilent Technologies, Santa Clara, USA) on a silica gel Lichrosorb Si-60 column (particle size 5 μm, 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain). α-Tocopherol separation was achieved with an isocratic elution of hexane/2-propanol (99:1; v/v) at the flow rate of 1 ml/mn. The fluorescence detector was set at 290 nm excitation wavelength and 330 nm emission wavelength. Identification and quantification of chromatographic peak were made by comparison with the response of α-tocopherol standard (CN Biosciences, La Jolla, CA). An external calibration curve was prepared for standard to calculate the amount of α-tocopherol present in the oil sample.

For the determination of FA composition, the methylesters were prepared by vigorous hand-shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of methanolic potassium hydroxide 2N (AOAC, 1990), and then 1 μl of the solution was injected into GC with a FID detector. A fused silica column (50 m length × 0.25 mm i.d.), coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor, Spain), was used. The carrier gas was helium, at a flow through the column of 1 ml/min. The temperatures of the injector and detector were set at 250 °C and the oven temperature at 210 °C. The identification of FA components was performed by comparison of their retention times with chromatogram provided by AOAC (1990).

2.6. Statistical analysis

Statistical analysis was performed by using the SPSS 10 statistical software (SPSS Inc., Chicago, IL). Descriptive analysis, one-way ANOVA, Duncan's comparison test, principal components was used. For each sample three determinations have been done. Differences at a confidence level of 95% were considered significant.

3. Results and discussion

3.1. Physico-chemical characteristics

The fruits of studied cultivars showed important differences in their physical and chemical characteristics (Table 1). *Meski* and *Picholine* cultivars had the highest fruit weight, which were appreciated by Tunisian consumers, while *Sayali* makes part of middleweight cultivars category (fruit weight ⩽4 g, IOOC, 2003). The ratio of pulp to stone (pulp/stone ratio) is very important to evaluate mass distribution between the pulp and the stone. Although, *Meski* had a higher fruit weight (6.4 g) than *Picholine* (4 g), but this later had the highest pulp/stone ratio (11.5) indicating that it had the greatest mass of pulp.

The quality of pulp is very important to evaluate if such fruit can be processed with sodium hydroxide treatment or not. Indeed, olive with smooth pulp was easily destroyed by sodium hydroxide solution that gives a bad product with an unpleasant aspect of pulp and a low nutritional value. *Sayali* fruit had a smooth pulp; however *Meski* and *Picholine* had a hard pulp especially at green stage. These physical parameters showed that *Picholine* cultivar had the best criteria of table olive (IOOC, 2003).

3.2. Effects of ripening and processing in α-tocopherol content

α-Tocopherol content, expressed in mg/kg of oil, increased during ripening of three cultivars (Fig. 1). The α-tocopherol amount moved from 36 to 77 mg/kg, from 42 to 130 mg/kg and from 75 to 116 mg/kg, respectively, for *Meski*, *Sayali* and *Picholine* from the 33rd to the 38th WAF. The three olive cultivars, which grown in the same area, differ significatively in α-tocopherol content. This difference probably linked to genotype characteristic and metabolic behavior of each cultivar. A linear trend between α-tocopherol amount and ripening stages was observed for all cultivars. Regression analysis showed a high correlation coefficient ($R = 0.995$; $R = 0.992$ and $R = 0.988$, respectively for *Sayali, Picholine* and *Meski*; $p < 0.05$). This linear correlation suggested that the biosynthetic of α-tocopherol was continuous during ripening of olive.

α-Tocopherol amount was positively correlated with oil content (expressed in % of dry weight), during the maturity of three cultivars (Fig. 2). The highest amounts of α-tocopherol and oil were detected in black fruits of three varieties, confirming their positive correlation. A linear trend between α-tocopherol and oil amounts was observed for all cultivars ($R = 0.976$; $R = 0.972$ and $R = 0.947$, respectively for *Sayali, Picholine* and *Meski*; $p < 0.05$). Considering the fact that α-tocopherol contributes to the stability of oil by protecting UFA against free radical

Table 1
Physico–chemical characteristics of fresh olive-fruits

Cultivar	Fruit weight (g)	Stone weight (g)	Pulp/stone ratio	Oil content (% d.w.)[b]	Pulp characteristics	Unsaponifiable content (% of lipids)
Meski	6.4[a] ± 2.3	1.7[a] ± 1.1	7.6[a] ± 1.4	33.5[c] ± 2.8	Hard[d]	1.9[c] ± 1.1
Sayali	4.0[a] ± 1.9	1.5[a] ± 1.2	7.0[a] ± 1.2	29.0[c] ± 2.7	Smooth[d]	1.9[c] ± 1.2
Picholine	5.0[a] ± 2.2	1.4[a] ± 1.2	11.5[a] ± 1.1	47.0[c] ± 2.4	slightly hard[d]	1.9[c] ± 1.1

[a] Means of 50 olive-fruits.
[b] % d.w., percentage of dried weight.
[c] Determined at complete maturity of fruits and data are means of three measurements.
[d] Determined at cherry stage.

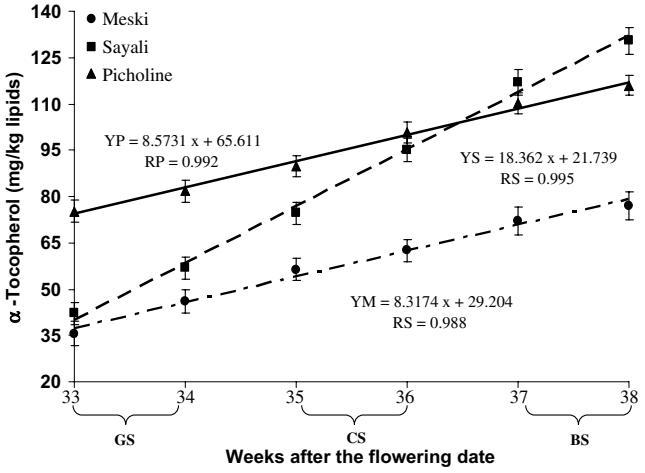

Fig. 1. Changes in α-tocopherol content during ripening of olive cultivars. Mean of three measurements (vertical line). GS, green stage; CS, cherry stage; BS, black stage.

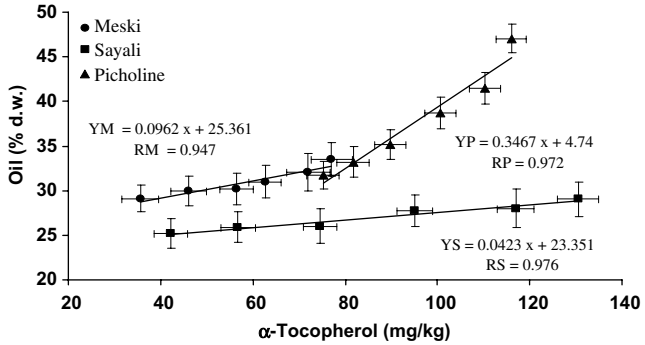

Fig. 2. Changes in oil (expressed in % of dry weight) and α-tocopherol contents during repining of olive cultivars. Means of three measurements (horizontal and vertical lines).

attack (Papadopoulos & Boskou, 1991), the increase of α-tocopherol content could be linked to the increase of UFA amount during ripening (Boskou, 1996).

α-Tocopherol concentration was negatively correlated with unsaponifiable amount (expressed in % of total lipids weight), during ripening of three cultivars (Fig. 3). In fact,

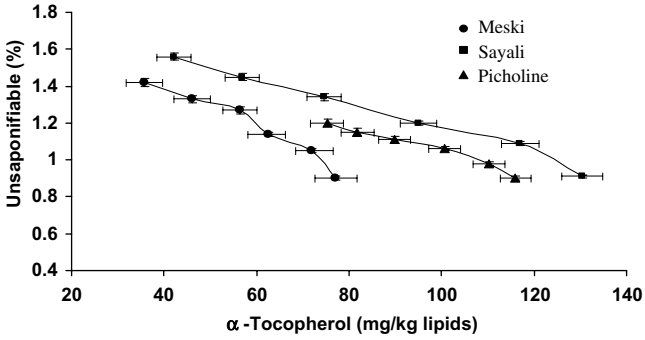

Fig. 3. Changes in unsaponifiable (expressed in % of total lipids) and α-tocopherol amounts during repining of olive cultivars. Mean of three measurements (horizontal and vertical lines).

α-tocopherol level increased while the total of unsaponifiable matter decreased. We suggested that this decrease in unsaponifiable content was linked to the decrease of others unsaponifiable compounds such as sterols (Harrabi et al., 2007; Salvador et al., 2001) and carotenoid pigments (Bertrán, Paz Aguilero, Del Rio, Sanchez, & Marting, 2005).

In processing, α-tocopherol content of processed olives decreased gradually when fruit changes from green to black (Fig. 4). This decrease was more pronounced in the black stage than in green one for all olive cultivars. α-Tocopherol level lost in brine was not dependent on

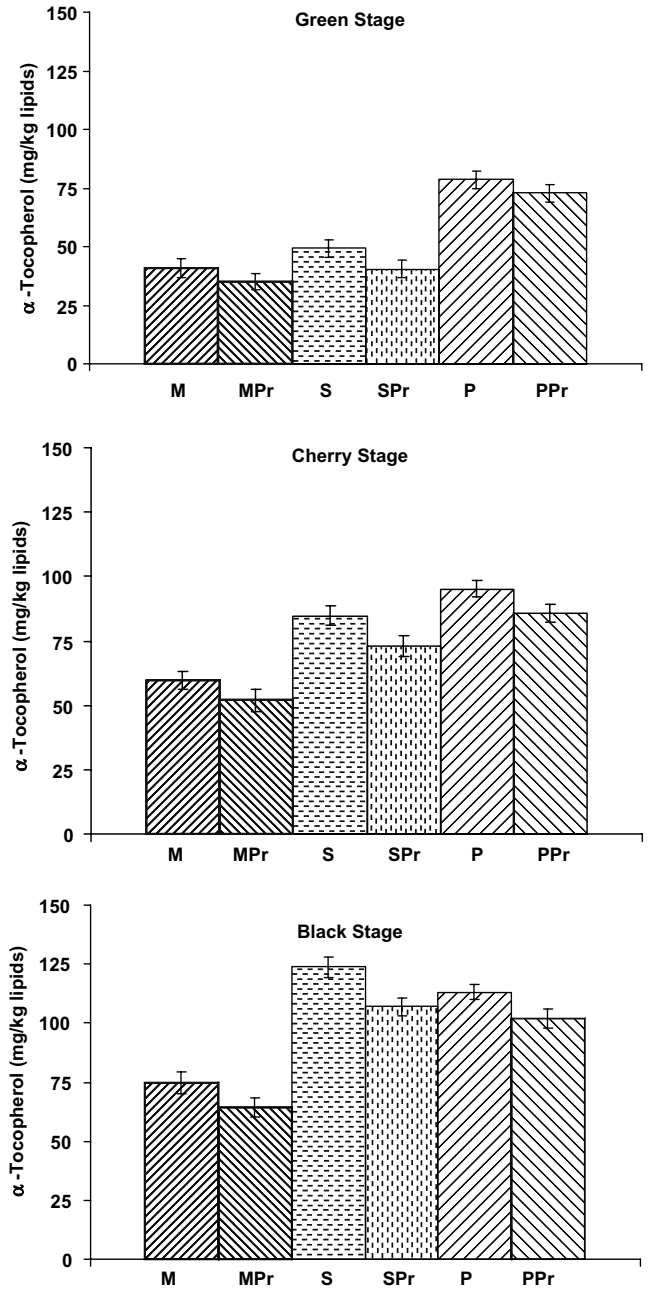

Fig. 4. Changes in α-tocopherol content after processing according to the anaerobic method. M, *Meski* fresh; MPr, *Meski* after processing; S, *Sayali* fresh; SPr, *Sayali* after processing; P, *Picholine* fresh; PPr, *Picholine* after processing. Mean of three measurements (vertical line).

its amount in fresh olive, but it was related to the quality of pulp fruit. This result can be explained by the fact that, in green stage the pulp was hard. This status decreases both the effect of sodium hydroxide on olive skin and the osmotic exchange between the pulp and the outside medium (brine). However, in black fruit, pulp was smooth and was easily destroyed by sodium hydroxide solution which accentuated the osmotic exchange between the pulp and the brine. The α-tocopherol level lost in brine was cultivars dependent. According to the processing with the Spanish style, α-tocopherol amount was less preserved in *Sayali* processed olive compared to *Meski* and *Picholine*.

The recommended level of α-tocopherol for having a good health is not mentioned in a clear way (Current Dietary Guidance for Healthy Nutrition). While, some research considered that α-tocopherol content above 0.6 mg/g of polyunsaturated fatty acids (PUFA) is adequate for healthy nutrition (Association, 2001; Li et al., 1999; American Health Truswell, 1995). So, in these conditions it is difficult to state that processing conserves or not the nutritional value of olives related to α-tocopherol content.

3.3. Effects of ripening and processing on FA content

Table 2 showed an important effect of variety on the major FA content (expressed as per cent of total FA), during ripening of three olive cultivars. Such differences in FA amount probably reflect the metabolic behavior of each cultivar in relation to genotype characteristic. Higher amounts of palmitic acid (C16:0) were found at green stage for all cultivars. Lower contents of oleic acid (C18:0) were observed at black stage for each olive variety. Although all olive cultivars were rich in UFA (79.4%; 82.3% and 83.1%, respectively, for *Meski*, *Picholine* and *Sayali* at cherry stage), their PUFA/SFA ratio was lower and irregular during ripening. In fact, PUFA/SFA ratios were 1.3; 1.1 and 0.4, respectively for *Meski*, *Picholine* and *Sayali* fresh olives at cherry stage.

After processing, FA contents of processed olives decreased in all cultivars (Table 3). FA content was less preserved in *Sayali* processed olive when compared to *Meski* and *Picholine*. This result can be explained by the fact that the pulp of *Sayali* fruit is very smooth which increases the moving of free FA into the brine. At cherry stage, the percentages of oleic and stearic acids lost in brine were 6.5% and 0.8%, respectively. This result may be linked to their percentages in fresh olives.

According to current dietary guidance for healthy nutrition, polyunsaturated fatty acids to SFA (PUFA/SFA) ratio above 1.5 is associated with good health (Ribarova et al., 2003). PUFA/SFA ratios of *Meski* and *Picholine* processed olives were 1.7 and 1.6, respectively at the cherry stage. These values showed that *Meski* and *Picholine* processed olives had a good health effect compared to fresh one (Table 3). However, in *Sayali* fruits neither fresh (PUFA/SFA = 0.4) nor processed olives (PUF/SFA = 0.6) had the nutritional value. Processing according the Spanish style provided the decrease of nutritional benefits of *Meski* and *Picholine* cherry olives.

3.4. Effects of processing in pulp and exocarp of fruit

The quality of fresh olive pulp differed from one stage to another for all cultivars. Indeed, at both green and cherry stages, fruits presented a hard pulp, while with the black one the pulp was smooth. These characteristics of pulp (hard and smooth) had a crucial role in the selection of the kind of processing method to use. Processing according to the Spanish style showed that the processed olives, at green and cherry stages, conserved their natural aspect and colour. However, processing of black olives using Spanish method damaged pulp and created lesions and undesirable spots on the pulp. These results suggested that

Table 2
Major fatty acids[a] composition (% of total fatty acids) and α-Tocopherol[a] content (mg/kg lipids) of studied cultivars at green, cherry and black stages

	Green stage			Cherry stage			Black stage		
	Meski	*Sayali*	*Picholine*	*Meski*	*Sayali*	*Picholine*	*Meski*	*Sayali*	*Pichline*
C16:0	18.9 ± 1.2	12.4 ± 1.7	14. 9 ± 1.5	17.7 ± 2.6	13.2 ± 2.4	15.2 ± 2.1	16.0 ± 2.2	11.0 ± 1.9	13.2 ± 1.6
C18:0	2.6 ± 1.1	2.6 ± 1.2	3.5 ± 1.3	2.8 ± 1.2	2.7 ± 1.1	2.9 ± 1.2	3.2 ± 1.2	2.7 ± 1.2	3.1 ± 1.4
C18:1	55.0 ± 1.3	78.5 ± 2.4	65.7 ± 2.1	53.0 ± 3.4	76.0 ± 3.8	61.7 ± 3.7	56.0 ± 3.7	77.4 ± 3.6	57.2 ± 3.8
C18:2	22.0 ± 1.2	4.7 ± 1.1	14.3 ± 1.8	24.5 ± 1.5	5.4 ± 1.2	18.5 ± 2.4	23.2 ± 2.4	5.9 ± 1.3	24.1 ± 1.5
C18:3	1.8 ± 1.1	1.9 ± 1.2	1.8 ± 1.1	1.9 ± 1.2	1.7 ± 1.1	2.1 ± 1.2	1.9 ± 1.1	1.7 ± 1.1	2.2 ± 1.1
$\sum$SFA[b]	21.5 ± 1.6	15.0 ± 1.1	18.4 ± 1.6	20.5 ± 1.4	15.9 ± 1.4	18.1 ± 1.1	19.2 ± 1.6	13.7 ± 1.2	16.3 ± 1.8
$\sum$UFA[c]	78.8 ± 3.2	85.1 ± 2.3	81.8 ± 3.8	79.4 ± 3.6	83.1 ± 3.5	82.3 ± 3.7	81.1. ± 4.2	85.0 ± 3.6	83.5 ± 3.3
$\sum$PUFA[d]	23.8 ± 2.4	6.6 ± 1.1	16.1 ± 2.4	26.4 ± 3.4	7.1 ± 1.2	20.6 ± 1.6	25.1 ± 3.7	7.6 ± 1.1	26.3 ± 1.6
$\sum$PUFA/$\sum$SFA[e]	1.1	0.4	0.9	1.3	0.4	1.1	1.3	0.6	1.6
α-Tocopherol	40.9 ± 3.9	49.2 ± 3.6	78.5 ± 3.5	59.5 ± 3.6	84.8 ± 3.8	95.2 ± 3.4	74.6 ± 4.6	123.7 ± 4.2	113.1 ± 3.3

[a] Mean of three measurements.
[b] Sum of major saturated fatty acids.
[c] Sum of major unsaturated fatty acids.
[d] Sum of major polyunsaturated fatty acids.
[e] Polyunsaturated fatty acids to saturated fatty acids ratio.

Table 3
Mean values of three measurements of major fatty acids composition (% of total fatty acids) before and after processing at cherry stage

	Black stage					
	Meski		*Sayali*		*Pichline*	
	BPr	APr	BPr	APr	BPr	APr
C16:0	17.7 ± 2.6	12.2 ± 1.4	13.2 ± 2.4	7.4 ± 1.3	15.2 ± 2.1	9.9 ± 1.6
C18:0	2.8 ± 1.2	2.0 ± 1.2	2.7 ± 1.1	1.9 ± 1.1	2.9 ± 1.2	2.2 ± 1.3
C18:1	53.0 ± 3.4	47.7 ± 2.3	76.0 ± 3.8	69.5 ± 2.6	61.7 ± 3.7	58.2 ± 2.8
C18:2	24.5 ± 1.5	23.2 ± 1.7	5.4 ± 1.2	4.5 ± 1.7	18.5 ± 2.4	17.3 ± 1.5
C18:3	1.9 ± 1.2	1.5 ± 1.1	1.7 ± 1.1	1.3 ± 1.1	2.1 ± 1.1	1.9 ± 1.1
$\sum$PUFA/$\sum$SFA[a]	1.3	1.7	0.4	0.6	1.1	1.6

BPr: before processing; APr: after processing.
[a] Polyunsaturated fatty acids to saturated fatty acids ratio.

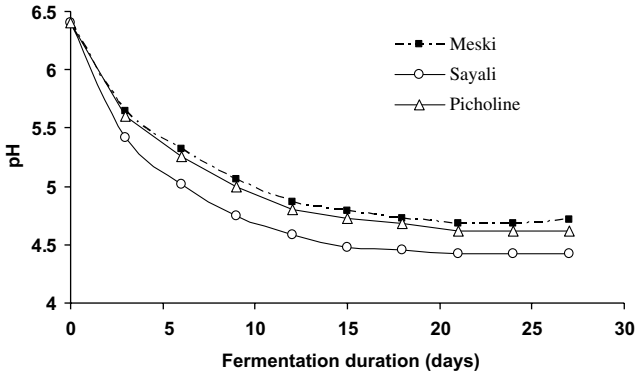

Fig. 5. Changes of pH in brine during the fermentation process of *Meski*, *Sayali* and *Picholine* cherry-table olives.

processing with the Spanish style was successfully used to green and cherry olives which guaranteed their natural aspect and colour. In contrast, black olives were recommended to be treated directly with brine or dry salt without recourse to alkali treatment.

3.5. pH and acidity development during fermentation

Changes of pH in brine showed the same profile in all cultivars during fermentation (Fig. 5). The highest rate of pH decrease occurred during the first week of fermentation. After the 10 days of fermentation, the drop of pH was much slower. This result could be linked to the increase of lactic acid produced by fermenting bacteria (Adams, 1990; Spyropoulou et al., 2001). Knowing that fermentation of three cultivars of table olives had been done in the same conditions, the difference between the pH values was related to the free FA lost in brine. As previously mentioned, the decrease of free FA in olives was more pronounced in *Sayali* fruit because they moved into brine leading to an increase of acidity and the lower value of pH in *Sayali* brine. However, in the case of *Meski* and *Picholine* cultivars, the decrease of pH was very similar and also these two cultivars have the same levels of free FA loosed in brine. These results seem to be related to the free FA released to the brine and the organic acids produced by bacteria lowers pH.

4. Conclusion

On the basis of our results, it can be stated that processing according to the Spanish style led to the decrease in both α-tocopherol and FA contents in all cultivars. It also led to increase the nutritional value of *Meski* and *Picholine* processed fruits. It will be interesting to mention that processing according to the Spanish style can be successfully used in cherry olives.

The knowledge about chemical and physical (aspect) characteristics in both fresh and processed table olives was very important to explore table olive cultivar which preserved quality and nutritional value of processed olives. These characteristics may contribute to have good nutritional values of this product in Mediterranean countries.

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