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Chemical parameters and antioxidant activity of turning color natural-style table olives of the Sigoise cultivar

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SUMMARY: A chemical characterization of turning color table olives of the Sigoise variety was made through their processing as natural-style. Polyphenols, sugars, tocopherols, fatty acids, and antioxidant activity in the olives were monitored throughout the elaboration process. Oleuropein, salidroside, hydroxytyrosol 4-glucoside, rutin, ligustroside and verbascoside showed a decrease of 16.90–83.34%, while hydroxytyrosol increased during the first months of brining. Glucose was consumed by 90% due to the metabolism of the fermentative microbiota. The tocopherol content remained stable during the process and only the α-tocopherol decreased. The fatty acids were not affected. The loss in antioxidant compounds resulted in a decrease in the percentage of DPPH radical inhibition from 75.91% in the raw fruit to 44.20% after 150 days of brining. Therefore, the turning color natural table olives of the Sigoise variety are a good source of bioactive compounds.

KEYWORDS: *Antioxidant activity; Polyphenols; Sigoise cultivar; Sugars; Tocopherols*

RESUMEN: ***Parámetros químicos y actividad antioxidante de aceitunas de mesa al estilo natural de color cambiante de la variedad Sigoise***. La caracterización química de las aceitunas en salmuera de color cambiante de la variedad Sigoise se ha estudiado durante el proceso de elaboración, en particular la concentración de fenoles, azúcares, tocoferoles, ácidos grasos y la actividad antioxidante. La concentración de oleuropeína, salidrósido, hidroxitirosol 4-glucósido, rutina, ligustrósido y verbascósido disminuyó un 16,90–83,34% durante el primer mes en salmuera. El 90% de la glucosa fue consumida debido al metabolismo de la microbiota fermentativa. El contenido en tocoferoles se mantuvo constante durante el proceso y solo disminuyó el α-tocoferol. Los ácidos grasos no se vieron afectados. La pérdida de actividad antioxidante se tradujo en una disminución del porcentaje de inhibición del radical DPPH de un 75,91% del fruto fresco a 44,20% después de 150 días en salmuera. A pesar de todo, la aceituna color cambiante de la variedad Sigoise en salmuera es una buena fuente de compuestos bioactivos.

PALABRAS CLAVE: *Actividad antioxidante; Azúcares; Polifenoles; Tocoferoles; Variedad Sigoise*

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1. INTRODUCTION

Table olives are elaborated from the fruit of olive tree (*Olea europaea* L.) and they are considered the most popular fermented vegetable in the Mediterranean countries. The production of table olives in Algeria is estimated at 293000 tons (IOC, 2020) and represents 10% of worldwide production. Olives are mainly composed of water (60-75%) and lipids (12-30%). The fruits also contain a small amount of proteins (1-2%) and sugars (2.6-6%). However, this composition depends on several factors, such as the type of cultivar and the ripening stage (Boskou *et al*., 2014). Among the minor compounds, polyphenols are the most relevant and represent the 1-3% of the fresh pulp weight. Oleuropein is the major phenolic compound and is responsible for the bitter taste, which makes the fresh fruit inedible (Kiai and Hafidi, 2014).

There are three main types of preparations which are widely used worldwide to reduce the bitterness: Spanish-style olives, Californian-style olives and natural olives or Greek-style. The two first use a diluted sodium hydroxide solution to hydrolyze the molecule of the oleuropein into non-bitter compounds and the last process consists of directly brining without using any chemicals.

Recently, there has been a growing interest in the characterization of some bioactive compounds in table olives, in particular polyphenols and tocopherols, and their relationship with the antioxidant activity which acts as a potent radical scavenger against free radicals (Sagratini *et al.*, 2013). The phenolic fraction in table olives can be influenced by many factors such as the olive cultivar (Medina *et al*., 2010), the degree of maturity (Sousa *et al*., 2014) and the de-bittering process applied (Ben Othman *et al*., 2009). Many studies have been carried out to assess the effect of the elaboration processes on these components (Bleve *et al*., 2015; Issaoui *et al*., 2011; Johnson *et al*., 2018; Romero *et al*., 2004a). The Greek-style elaboration process showed higher total phenolic compounds than the Spanish or Californian-style processes and hydroxytyrosol was the predominate compound in all three styles of commercial olives (Johnson *et al*., 2018). Greek-style processing alos better preserved the tocopherol content than the Spanish-style elaboration (Hassapidou *et al*., 1994).

Despite all these studies, only a few works have been focused on turning color table olives. Changes in the polyphenol content in the turning color Sigoise variety during naturally brined olive processing was studied (Mettouchi *et al*., 2016). It was found that turning color Manzanilla olives in brine showed more polyphenol concentration than those elaborated as Spanish-style (Romero *et al*., 2004b). The sugar and polyol compositions in natural olives at the cherry stage of ripening were studied (Marsilio *et al*., 2001), concluding that the determination of these components is important to optimize table olive processing.

According to the "Trade Standard Applying to Table Olives" (IOC, 2004), "natural olives could be "green olives, turning color or black olives placed directly in brine, where they undergo complete or partial fermentation, preserved or not by the addition of acidifying agents". The maturity of the fruit affects the phenolic concentration and may contribute to significant consequences concerning the technology and quality of table olives. The Sigoise variety, which is grown mainly in the north-west of Algeria (Sig region) is the most popular variety used in Algeria as fermented green olives (Kacem and Karem, 2006). In this work, the elaboration of turning color table olives of the Sigoise cv. according to the natural process was studied to characterize the chemical composition and evaluate changes in polyphenols, sugars, tocopherols, fatty acids and antioxidant activity.

2. MATERIALS AND METHODS

2.1. Processing and sampling

Olives of the Algerian Sigoise variety (average weight 3.5 g) were harvested at the turning color stage during the 2015-2016 season and placed in plastic tanks (30 L capacity). The olives were processed according to the natural style elaboration. An initial brine of 11% NaCl was used to cover the fruits, which were then left at ambient temperature to follow spontaneous fermentation for five months. Samples were withdrawn periodically at 60, 120 and 150 days of brining until the brine reached a pH of 4.3. Fresh fruits were also collected at the time of harvesting. Samples were stored frozen at -20 ºC until analysis.

2.2. Determination of physical properties, moisture and oil content in fruits

Fifteen olives from each sample were weighed. Length and diameter were measured using a digital calliper (150 mm (6'')) (Stainless Hardened). The

flesh/stone ratio was estimated to characterize the shape and size of the olives. The pH of the brines was monitored during the elaboration process with a pH meter (Crison Basic 20).

Fruit moisture was determined (Tovar *et al.,* 2002). Five grams of fresh pulp were desiccated at 105 °C until constant e weight.

Oil was extracted from dried olives with hexane in a Soxhlet apparatus for 6 h at 45 ºC (ECC) No 2568/91 of July (1991). The solvent was removed by a rotary evaporator, and the oil was weighed and stored at 4 ºC until further analysis. The results were expressed as percentage of dry weight.

2.3. HPLC analysis of phenolic compounds

The phenolic compounds were extracted from the olive pulp with dimethylsulfoxide (DMSO) as described by Susamci *et al*. (2017). 1.5 g of freeze-dried olive pulp were homogenized with 30 mL of DMSO and centrifuged at 6000g after 30 min of contact. Then, an aliquot of 0.25 mL of supernatant was mixed with 0.5 mL of DMSO and 0.25 mL of 0.2 mM syringic acid in DMSO (internal standard). The mixture was filtered through a 0.22 µm pore size nylon filter, and an aliquot of 20 µL was injected into the chromatograph. The analytical column, mobile phases, gradient and equipment were the same as those used by Susamci *et al.* (2017). The wavelengths selected for phenolic compounds were 280 nm.

2.4. HPLC analysis of sugars

The sugars were extracted from the olive pulp as described elsewhere (Medina *et al*., 2007) with some modifications. One gram of freeze-dried olive flesh was mixed with 20 mL of boiling water and 2 mL of sorbitol (7.5%, w/v) as internal standard, vortexed for 1 min and kept in an ultrasonic bath for 3 min. The mixture was centrifuged at 9000 g for 5 min and filtered through filter paper under vacuum into a 50 mL flask. A second extraction was repeated with another 20 mL of hot water and made up to volume. 2 mL of the filtered solution (0.22 µm pore size nylon filter) were put into contact with 1 g of the acidic resin Amberlite IR-120 plus 1 g of the basic resin Amberlite IRA-93, shaken for 30 min, and centrifuged at 9000 g for 3 min. An aliquot of 20 µL was injected into the chromatograph. The analytical column, mobile phases, gradient and equipment were the same as those used by Medina *et al*. (2007).

2.5. HPLC analysis of tocopherols

After cold extraction of the oil in a laboratory mill (Levi-Dilon-Lerogsame), the tocopherol composition was evaluated using an HPLC system, analytical column, mobile phases, gradient and equipment as described by Rovellini *et al*. (1997). The wavelengths selected for tocopherols were 292 nm. The different isomeric forms were identified by comparing other vegetable oils with typical tocopherol content distribution. The quantification was conducted using an external calibration solution of alpha-tocopherol in acetone (0.01 mg/mL).

2.6. Fatty acid composition

The fatty acid methyl esters (FAME) were prepared in a solution of 2 N methanolic potassium hydroxide according to the method described (Commission Delegated Regulation (EU) 2016/2095 of 26 September 2016 amending Regulation (EEC) No 2568/91) and analyzed by gas chromatography. An Agilent 7890 gas chromatograph (Agilent, Germany) equipped with capillary column HP88 (Agilent, Germany) 112-88177 (100 m, 0.25 mm, 0.20µm), a flame ionization detector (FID) and a split/splitless injector was used. The oven temperature was programmed as follows: from 60 °C (1 min) to 165 °C to 10 °C/min and held for 1 min; then heated to 225 at 2 °C/min and finally an isothermal was used for 25 min. Helium was used as carrier gas.

2.7. Antioxidant activity

2.7.1. Radical scavenging activity of methanolic extracts against DPPH radical

The extraction of phenolic compounds from the pulp was performed according to the method described by McDonald *et al*. (2001). Ten grams of dried pulp were mixed with 50 mL of methanol/water (80:20, v/v) for 20 minutes. The mixture was centrifuged at 3000 rpm for 5 minutes. Then, the residue was extracted twice, and supernatants were combined and washed with hexane to eliminate the oil. The extract was filtered through a 0.45 µm pore size filter. An aliquot of 0.5 mL of methanolic extracts was reacted with 3.9 mL of a methanolic solution of DPPH radical (0.1 mM). The mixture was incubated for 30 min in the dark, and the

absorbance was measured at 515 nm. The radical inhibition was calculated according to the following formula:

% inhibition = [(absorbance of control - absorbance of the sample)/absorbance of control] x100 (Boskou *et al*., 2006).

The antiradical activity was expressed in mg of gallic acid equivalents (GAE)/100g of dry weight (DW). The EC50 value, which represents the concentration of sample required to inhibit 50% 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined for each extract.

2.7.2. Reducing power

The determination of reducing power was carried out (Zhan *et al*., 2006). An aliquot of 2.5 mL of the methanolic extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) were added, and the mixture was centrifuged at 3650 rpm for 10 min. 5 mL of supernatant were mixed with the same volume of distilled water, and 1 mL of ferric chloride (0.1%) and the absorbance was measured at 700 nm. The results were expressed as mg of quercetin equivalents (QE)/100g DW.

2.8. Statistical analysis

Three tests were performed and expressed as mean values (n=3) for each analysis. Statistica software 10.0 (StatSoft, Inc., Tulsa, OK, USA) was used for data analysis. Statistical comparisons of the mean values for each experiment were performed by the least significant difference Newman–Keuls test and significance was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physical characteristics, moisture and oil content

Table 1 summarizes the main characteristics of olive drupes. The flesh-to-pit ratio is vital to evaluate the mass distribution between the flesh and the stone (Sakouhi *et al*., 2008). A proper appreciation requires drupe with flesh/pit equal to 5 (Rallo *et al*., 2018). Turning color olives of the Sigoise cultivar with a weight of 3.47 g and a flesh/stone ratio of about 5.09 can be considered appropriate for table olive processing. The olives have an oval shape, and the length/diameter ratio is greater than 1.

The evolution of the pH of the brines during the fermentation of table olives showed a significant decrease from 6.65 initially to 4.2 after 150 days. A critical drop was noticed after 60 days of fermentation. This trend is due to the conversion of carbohydrates into organic acids, mainly by LAB metabolism. Also, the hydrolysis of oleuropein, which is decomposed by endogenous and bacterial enzymes in sugars and simple phenols, such as elenolic acid, may contribute to the drop in pH (Kiai and Hafidi, 2014).

The humidity varied between 52.74 and 54.70%; whereas oil content varied between 42.98 and 44.61%. Sigoise turning color table olives recorded a higher rate of oil compared to those reported for the Tunisian table olives (Meski) elaborated according to natural style (Issaoui *et al*., 2011). There was a non-significant effect of processing on all the main characteristics of table olives except for pH.

Table 1. Physicochemical parameters in turning color table olives of the Sigoise variety during natural-style processing.

Time (days)	Weight (g)	Flesh to pit ratio	Length/diameter ratio	pH	Moisture content (%)	Oil content (% Dry weight)
0	3.49 ± 0.49	5.02 ± 0.22	1.32 ± 0.06	6.65 ± 0.05a	55.86 ± 0.53	44.61 ± 1.45
60	3.51 ± 0.74	5.05 ± 0.19	1.34 ± 0.06	5.25 ± 0.05b	52.75 ± 0.67	42.98 ± 2.19
120	3.53 ± 0.63	5.01 ± 0.13	1.34 ± 0.1	4.45 ± 0.05c	54.69 ± 0.33	44.34 ± 1.18
150	3.47 ± 0.34	5.09 ± 0.35	1.35 ± 0.07	4.20 ± 0.05d	53.72 ± 0.04	43.86 ± 1.33

Results are given as mean ± standard deviation of triplicate analyses. No significant differences were found among samples except for the pH, which are indicated by different superscript letters using the Newman–Keuls test ($p < 0.05$).

3.2. Phenolic compounds

Polyphenols are considered to be the most antioxidant substrates in table olives. Still, some of them, in particular the oleuropein, confer a bitter taste which is not accepted by consumers and their removal is necessary (Boskou *et al*., 2006). The phenolic composition in the olives was studied in the raw fruit and throughout processing (Table 2). Hydroxytyrosol 4-glucoside and oleuropein were identified as the major polyphenols in the raw olive fruit with a concentration of 31867.9 and 27730.3 mg/kg DW, respectively, followed by verbascoside, rutin, hydroxytyrosol, ligustroside and salidroside in minor concentrations. These results are in accordance with those found previously (Romero *et al*., 2004b).

Noticeable decreases in oleuropein, hydroxytyrosol 4-glucoside, rutin and ligustroside were observed after 150 days of brining, reaching concentrations of 10891.7, 10860.0, 310.1 and 252.1 mg/kg DW, respectively. Conversely, hydroxytyrosol and salidroside increased after 60 days and decreased at the end of processing to 1617.8 and 303.8 mg/kg respectively. Comselogoside did not show significant differences during processing. The same trend was observed by Sousa *et al*. (2014) which showed a decrease in oleuropein and increase in hydroxytyrosol in samples harvested at different stages of maturation. Moreover, several authors indicated that table olives processed according to the natural style underwent a significant decrease in oleuropein, which is followed by an increase in hydroxytyrosol as the main hydrolysis product during the first days of brining (Issaoui *et al*., 2011; Johnson *et al*., 2018; Ramirez *et al*., 2016).

The total polyphenol content in fresh olives was initially 67335.4 mg/kg. This amount was significantly reduced during processing, reaching 26585.5 mg/kg after 150 days of brining, which corresponds to a decline in total polyphenols of 60.52% (Table 2). Similar total polyphenol concentrations for the Picholine variety in both raw and processed fruit were found (Issaoui *et al*., 2011). Likewise, Sigoise cv. showed a higher content in phenolic compounds compared to Meski and Manzanilla cultivars. The study conducted by Johnson *et al*. (2018) on levels of phenolic compounds in Spanish-style green (SP),

Table 2. Concentration (mg/kg of dry weight) of the phenolic compounds in turning color table olives of the Sigoise variety during natural-style processing.

	Time (days)			
	0	**60**	**120**	**150**
Hydroxytyrosol	1291.8 ± 84.1^{b}	2456.9 ± 178.9^{a}	1679.6 ± 95.8^{b}	1617.8 ± 152.2^{b}
Hydroxytyrosol 4-glucoside	31867.9 ± 1595.4^{a}	28753.7 ± 748.5^{b}	16415.4 ± 99.0^{c}	10860.0 ± 867.2^{d}
Salidroside	684.3 ± 22.7^{b}	916.8 ± 3.0^{a}	429.3 ± 40.8^{c}	303.8 ± 25.1^{d}
Verbascoside	2812.9 ± 170.0^{a}	3854.6 ± 117.8 ^{a}	1952.9 ± 126.0 ^{c}	2337.5 ±168.6 ^{b}
Oleuropein	27730.3 ± 702.9 ^{a}	21128.2 ± 655.1 ^{b}	14049.0 ± 526.5 ^{c}	10891.7 ± 580.2 ^{d}
Comselogoside	20.6 ± 9.5 ^{a}	17.2 ± 4.0 ^{a}	19.8 ± 4.8 ^{a}	12.3 ± 8.3 ^{a}
Rutin	1861.1 ± 82.1 ^{a}	771.2 ± 69.1 ^{a}	389.3 ± 60.9 ^{c}	310.1 ± 60.4 ^{c}
Ligustroside	1066.5 ± 126.4 ^{a}	531.4 ± 20.3 ^{b}	402.6 ± 17.4 ^{c}	252.1 ± 35.5 ^{d}
Sum of polyphenols	67335.4 ± 2233.4 ^{a}	58430.2 ± 1082.1 ^{b}	35338.6 ± 765.5 ^{c}	26585.5 ± 1106.0 ^{d}

Results are given as mean ± standard deviation of triplicate analyses. Significant differences in the same row are marked with different superscript letters using the Newman–Keuls test ($p < 0.05$).

Californian-style black ripe (CA), and Greek-style natural fermentation, showed that olives in brine had the highest concentrations; whereas CA olives had the lowest level.

It is well-known that the diffusion of polyphenols between fruits and the water phase takes place when olives are directly brined and soluble components move from olive to the surrounding solution (Poiana and Romeo, 2006). This diffusion can occur more rapidly if the cellular structure of the fruit becomes naturally soft. It was reported that the de-bittering process occurs by the breakdown of the oleuropein into non-bitter products by the action of the activity of endogenous enzymes, esterase and β-glucosidase during the first month of brining followed by slow chemical hydrolysis throughout the rest of the storage (Ramirez *et al*., 2016). The acidic conditions of the brine can also favor the chemical hydrolysis of oleuropein (Medina *et al*., 2008). Also, an oleuropeinolytic activity has been reported for different yeast and lactic acid bacteria strains, which are responsible for fermentation in natural table olives (Bonatsou *et al*., 2015; Ramírez *et al*., 2017).

3.3. Sugars

The sugar content in olives is the most important fermentative substrate for the growth of the microorganisms which are responsible for fermentation during the natural style elaboration. The soluble sugars are transformed by microorganisms into organic acids as the product of the fermentation and second metabolites responsible for the desirable organoleptic characteristics in the final product. As shown in Figure 1, the main sugars in the raw olive fruits are glucose, mannitol, fructose and sucrose with concentrations of 29.2, 10.5, 5.2 and 1.5 g/kg, respectively. The sugar content registered a significant decrease after 150 days of brining with a glucose degradation rate of 91.05% and lower for the mannitol and fructose (74.57 and 74.76%, respectively). Sucrose was consumed in the first 60 days of brining. A decrease in sugar content has also been noted for different cultivars elaborated in the natural style (Bianchi, 2003; Issaoui *et al*., 2011). Likewise, these results are in agreement with those noted (Bleve *et al*., 2015) for Taggiasca natural table olives but with a higher degradation rate for fructose (92.28%) than for glucose (83.55%).

3.4. Tocopherols

The study on tocopherols contributes to empowering the nutritional value and the biological properties like the antioxidant capacity of table olives (Sakouhi *et al*., 2008). The main tocopherol present in the oil fraction of raw olives was α-tocopherol with 133.8 mg/kg. β, γ, and δ-tocopherol were present at in small amounts of 0.4, 4.2, and 1.5 mg/kg, respectively (Table 3). A significant decrease was observed for α-tocopherol throughout processing and a loss of

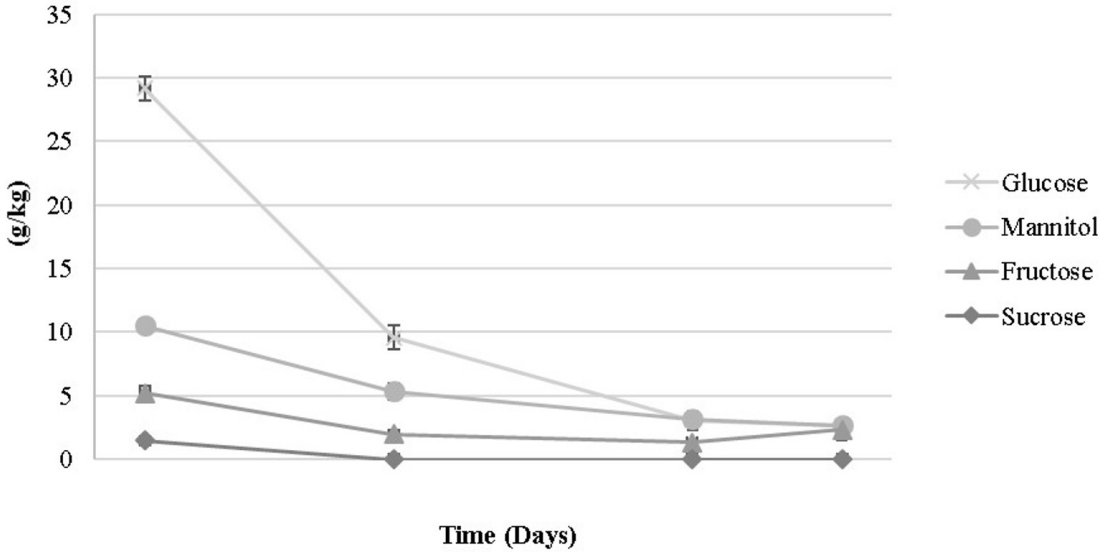

FIGURE 1. Concentration of sugars (g/kg) in turning color table olive pulp of the Sigoise variety during natural-style processing. Results are given as the mean of triplicate analyses. Bars indicate standard deviation.

TABLE 3. Concentration of tocopherols (mg/kg) and fatty acids (%) in turning color table olives of the Sigoise variety during natural-style processing.

	Time (days)			
	0	**60**	**120**	**150**
α-tocopherol	133.8 ± 3.9[a]	131.8 ± 3.5[ab]	122.8 ± 0.5[b]	122.4 ± 0.6[b]
β-tocopherol	1.5 ± 0.1[a]	1.5 ± 0.1[a]	1.4 ± 0.1[a]	1.4 ± 0.1[a]
γ-tocopherol	4.2 ± 0.4[a]	4.3 ± 0.4[a]	4.5 ± 0.2[a]	4.5 ± 0.1[a]
δ-tocopherol	0.4 ± 0.0[a]	0.5 ± 0.1[a]	0.3 ± 0.0[a]	0.4 ± 0.1[a]
Sum of tocopherols	139.8 ± 4.5[a]	138.1 ± 3.7[a]	128.9 ± 0.4[b]	128.8 ± 0.7[b]
C14:0	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C16:0	9.09 ± 0.04	9.16 ± 0.02	9.12 ± 0.01	9.30 ± 0.20
C16:1	0.64 ± 0.00	0.64 ± 0.01	0.64 ± 0.00	0.66 ± 0.02
C17:0	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.00
C17:1	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
C18:0	2.84 ± 0.00	2.87 ± 0.03	2.84 ± 0.02	2.87 ± 0.01
C18:1	75.90 ± 0.04	75.72 ± 0.04	75.94 ± 0.03	75.67 ± 0.18
C18:2	9.89 ± 0.04	9.98 ± 0.03	9.86 ± 0.04	9.86 ± 0.04
C18:3	0.75 ± 0.00	0.76 ± 0.00	0.75 ± 0.00	0.76 ± 0.00
C20:0	0.32 ± 0.01	0.32 ± 0.00	0.31 ± 0.01	0.31 ± 0.01
C20:1	0.33 ± 0.00	0.33 ± 0.00	0.32 ± 0.00	0.32 ± 0.01
C22:0	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.01
C24:0	003 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
trans C18:1	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
trans C18:2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
trans C18:3	0.00 ± 0.00	0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00
Sum of *trans*	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01

Results are given as mean ± standard deviation. Significant differences in the same row are marked with different superscript letters ($p < 0.05$). No significant differences were found for fatty acids among samples using the Newman–Keuls test ($p < 0.05$). ND means not detected.

7.88% was recorded after 150 days of brining, while the other isomers did not show significant changes.

The turning-color table olives of the Sigoise cv. registered higher tocopherol content than those found for Italian varieties (Sagratini *et al*., 2013) with maximum concentrations of 90 mg/kg in several samples of table olives elaborated by different processing methods. Similarly, Sakouhi *et al*. (2008) recorded higher tocopherol levels in three cultivars (Meski, Sayali and Picholine) at a cherry stage of ripeness than green ones, but still lower than the Sigoise variety. Hassapidou *et al*. (1994) reported that the natural-style elaboration did not affect the α-tocopherol contents in Conservolea or Kalamata black olives; however, the lye treatment used in the Spanish-style processing caused a reduction in α-tocopherols (Boskou *et al*., 2014).

3.5. Fatty acids

The fatty acid composition (Table 3) showed that oleic acid (C18:1) was the major fatty acid in the olive flesh (75.90% of the total). Linoleic (C18:2) and palmitic (C16:0) acids were present in similar amounts

of 9.89 and 9.09%, respectively. The variations related to processing were insignificant, which is in agreement with the results of many authors (Issaoui *et al*., 2011; Sakouhi *et al*., 2008). The stability of the fatty acids in processed olives could be related to their nature and the protective action of antioxidants. Oleic acid and tocopherols were present in considerable amounts in our variety. The *trans* forms of linoleic and linolenic acids were not detected, whereas *trans*-oleic acid (C18:1t) had a value (0.02%) which was below the limit values requested by the Commission Regulation (EU); ≤ 0.05 (Commission Delegated Regulation (EU) 2016/2095 of 26 September 2016 amending Regulation (EEC) No 2568/91). The preservation of essential fatty acids can also be explained by the firmness of olives and the non-use of alkaline treatment (Rallo *et al*., 2018; Jiménez *et al.,* 1997).

3.6. Antioxidant activity

In this study, antioxidant activity was evaluated by two different chemical assays, the scavenging effect on DPPH free radicals and the reducing power (Figure 2). The raw fruit recorded high values for antioxidant scavenging activity (40654.13 mg GAE/kg) and reducing power (12296.79 mg QE/kg). The inhibition rate of the DPPH radical and EC50 was 75.81% and 3.36 mg/mL, respectively. The antioxidant activity

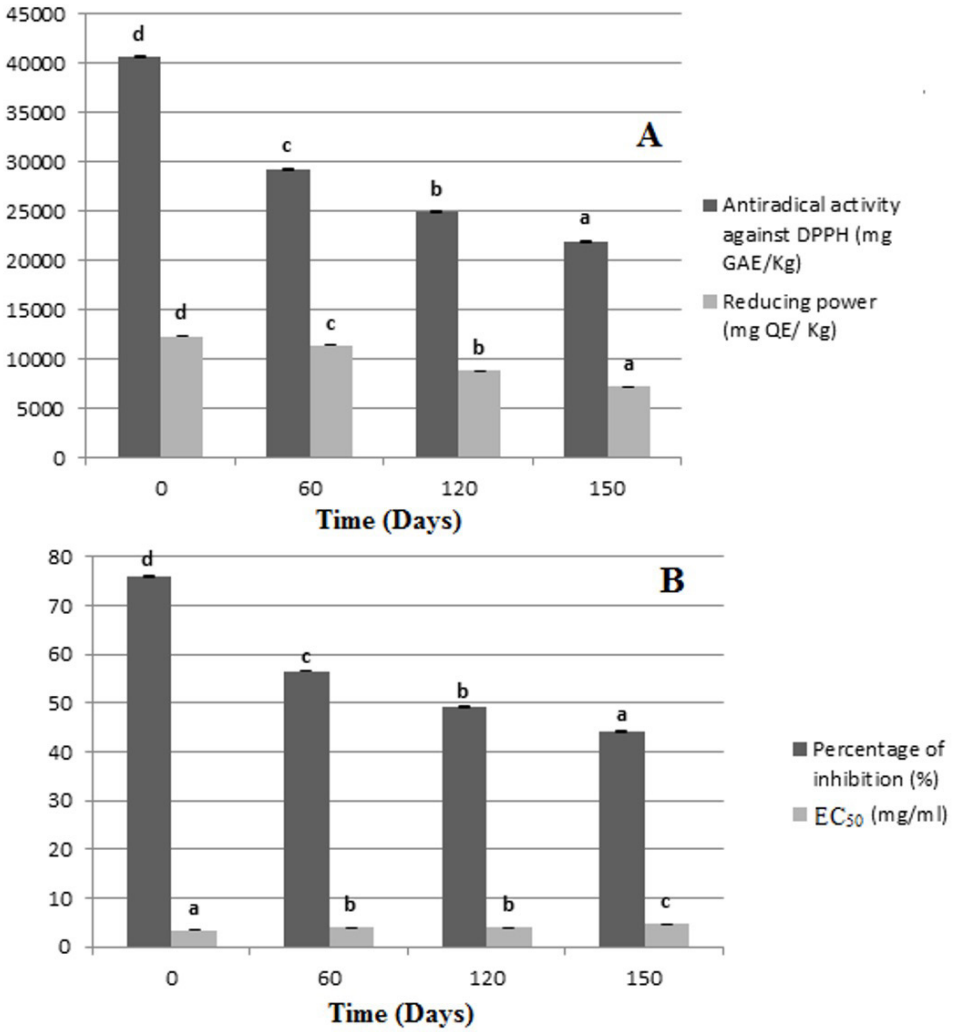

FIGURE 2. Antioxidant activity, reducing power (Panel A), percentage of inhibition and EC50 (Panel B) in turning color table olives of the Sigoise variety during natural-style processing. Results are given as mean of triplicate analyses. Significant differences are marked with different superscript letters using the Newman–Keuls test ($p < 0.05$). Bars indicate standard deviation. GAE means gallic acid equivalents. QE means quercetin equivalents.

decreased significantly throughout processing. The DPPH radical scavenging and reducing power decreased to 21917.71 mg GAE/kg and 7176.09 mg QE/kg, respectively, with an inhibition rate of 44.20%. The antioxidant activity showed a highly significant correlation with phenolic compounds (r = 0.99); whereas the effective concentration EC50 of the extracts was negatively correlated (r = -0.91). According to several authors, there is a linear correlation between the total polyphenol content in table olives and their antioxidant activity (Ben Othman *et al*., 2009; Romero *et al*., 2004b; Sousa *et al*., 2014). Mettouchi *et al*. (2016) reported a similar behavior for the loss in reducing power but with the influence of environmental conditions for the same variety. Indeed, Sigoise from the Sig region exhibited a low reducing power compared to Sigoise from the Tazmalt region. The differences could be explained by the effectiveness of each phenolic component in the fruit composition. So far, the antioxidant activity depends on the concentration of the polyphenol profile. Furthermore, Ben Othman *et al*. (2009) monitored the evolution of phenolic compounds during the natural table olive elaboration of the Chétoui cultivar at three degrees of ripeness (green, turning color and black) and they found a decrease in the antioxidant activity by 60–72%, with the turning color olives presenting an intermediate value. The Sigoise cultivar showed a lower reduction in the antioxidant capacity (46.09%) due to a more moderate loss in phenolic compounds.

4. CONCLUSIONS

This study has characterized the main compounds, in particular, polyphenols, sugars, fatty acids and tocopherols, in the turning color table olives elaborated as natural-style of the Sigoise cultivar. The total polyphenol content decreased as the process progressed, except for the hydroxytyrosol, which increased at the beginning of brining and then decreased. There was also a consumption of sugars, especially glucose, which is typical of the fermentation process. The concentration of fatty acids was not affected by the elaboration process without significant differences throughout the process. The tocopherol concentration remained constant during the processing, and a decrease was only observed for α-tocopherols after 150 days of brining. The losses in the latter and other phenolic compounds came as a consequent reduction in the antioxidant activity. Therefore, the turning color natural table olives of the Sigoise variety have an outstanding nutritional value and constitute an excellent source of antioxidant compounds that can exert beneficial properties to consumers.

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