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4 **Quality of lipid fraction during Spanish-style table olives processing of**
5 **Sigoise and Azzeradj cultivars.**

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18 **HIGHLIGHTS**

19 - The effect of different steps of Spanish-style processing on lipid fraction was studied for two
20 Algerian cultivars.

21 - Most of the variance of the quality parameters and fatty acids during processing was due to
22 cultivars.

23 - The alkali treatment and washing steps were more detrimental for phenolic compounds than
24 fermentation

25 - Antioxidant activity of the methanolic extract of oil was more affected than those of oil.

ABSTRACT

This work aimed to study the effect of different steps of Spanish-style processing (raw olives, lye treatment, washing, and fermentation) on the oil quality index (Acidity, peroxide value (PV) and extinction coefficient UV at 232 nm and 270), fatty acids, phenols, tocopherols, hexanal and nonanal compounds, and antioxidant activity of the lipid fraction of green Sigoise and Azzeradj cultivar. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) was used to discriminate the samples according to cultivars and elaboration phases. Most of the variance of the quality parameters and fatty acids was due to cultivars and less for processing.

After processing, a substantial loss in the total and individual phenolic compounds was recorded (79.25 % and 67.58 % for Azzeradj and Sigoise, respectively), but the most significant reduction occurred at lye treatment and washing step rather than the fermentation. Tocopherols were less affected than the phenolic compounds. The antioxidant activity against DPPH radicals of the total lipid fraction of olives was less affected than that of its methanolic extract alone.

Keywords: Table olives, Spanish-style, Fatty acids, tocopherols, phenolic compounds, antioxidant activity.

1. Introduction

The olive tree (*Olea europaea* L.) is one of the most important fruit trees in Mediterranean countries. Its products, olive oil and table olives, are important components of the Mediterranean diet. Algeria is one of the major olive producing countries; olive trees ranked first amongst the fruit trees (Algerian Ministry of Agriculture). Table olive production has undergone a remarkable evolution in recent years to reach 293.000 tons (campaign 2016/2017), which represent 10% of the world production (IOOC, 2019).

The edible pulp of the olive fruit is mainly composed of water (70–75 %) and lipids. The oil content in the olive fruit ranges from 14 to 30 %, depending on the cultivar and the ripening stage (Bianchi, 2003). Table olives provide many health-protective nutrients, such as oleic acid, α -tocopherol, and numerous antioxidant polyphenols. They contain also high dietary fiber, important minerals and microelements, phytosterols, triterpenic acids (maslinic and oleanolic acids), squalene and minor quantity of beta-carotene, pantothenic acid, and vitamin B1 (Lanza, 2012; Boskou, Camposeo, & Clodoveo, 2015). The health benefits associated with the consumption of table olives concern many systems: cardiovascular, digestive, respiratory, immune, and nervous (Boskou, 2017). Raw olives are bitter and non-edible due to the high content of oleuropein, a secoiridoid phenolic compound. Table olives are consumed after removing their bitterness by several methods. There are three main types of commercial table olives: Spanish-style green olives, Greek-style natural black olives, and California-style black ripe olives (Boskou et al., 2015).

During processing, physicochemical changes occur in olives. In the Spanish style, treating olives with a diluted aqueous solution of NaOH and fermentation in brine can lead to several

changes in the composition of the fruit. It was thought that the localization of the oil droplets in the interior of the cell in the flesh protected it from the various aqueous solutions to which the olives were subjected during treatment (**Garrido-Fernández, Fernández-Díez, & Adams, 1997**).

The fat content and its composition in table olives have been studied (**López, Montano, García, & Garrido, 2006**) and have been found to be similar to olive oil (**Aparicio, & Harwood, 2003**). In general, the green Spanish-style table olives fat preserves their original natural nutritive and healthy characteristics; most of the variance in the results for fatty acids, triacylglycerols and quality parameters are related to variety and only and little to the treatment (**Lopez-Lopez, Cortés-Delgado, & Garrido-Fernandez, 2015**). Conversely, a significant increase of all the oxidative and hydrolytic indexes of the lipid fraction during natural-style processing of table olives was reported (**Pasqualone, Nasti, Montemurro, & Gomes, 2014**). In addition, the processing of olives as ripe olives showed that acidity, peroxide value, K_{270} and ΔK increased during storage/fermentation; most of the fatty acids and triacylglycerols also underwent changes during processing (**Lopez-Lopez, Rodríguez-Gomez, Cortes-Delgado, Ruíz-Mendez, & Garrido-Fernandez, 2009a**).

Other studies have shown that ripe olive preparation may affect the minor components of the lipid fraction. The changes in α -tocopherol of Tunisian autochthonous cultivars processed according the green Spanish-style depend on the cultivar (**Sakouhi et al., 2008**). Unsaponifiable matter, sterols and triterpenic alcohol contents in table olives were slightly affected by processing (**Lopez-Lopez, Rodríguez-Gomez, Cortes-Delgado, Ruíz-Mendez, & Garrido-Fernandez, 2009b**). Also, the NaOH treatment employed to debitter black and green olives reduced the concentrations of triterpenic acid (**Romero et al., 2010**). Recently, minor-component transformations (polar compounds, sterols, fatty alcohols, triterpenic dialcohols, waxes, and alkyl esters) in fat was assessed during the green Spanish-style table

olive processing (López-López, Cortés-Delgado, & Garrido-Fernández, 2018), and concluded that most of the variability was due to differences between cultivars but not to processing .

The table olive processed according to the Spanish style is one of the most important fermented products in Algeria. Preservation of olive fat quality is essential due to its important role in human health. The aim of this work was to assess the effect of Spanish-style processing on oil quality index, the major fraction (fatty acids), the minor fraction (phenolic, tocopherols, volatiles compounds) and antioxidant activity of the lipid fraction of green Sigoise and Azzeradj cultivars. Principal component analysis and hierarchical clustering were used to study the behavior of the samples according to cultivars and production phases.

2. Material and methods

2.1. Sampling and olive processing

The olives of the Azzeradj (from Bejaia: North-east of Algeria) and Sigoise (from Mascara: North-west of Algeria) cultivars harvested at green maturation stage were used for the experimentation. Olives from each cultivar were processed according to the green Spanish-style elaboration. The process consisted of treating the olives with 2 g/100 mL NaOH solution until the alkali reached 2/3 of the flesh. Then the fruits were washed with tap water for 18 h, brined in a 10 g/100 mL NaCl solution (regardless of cultivar), and left to follow spontaneous fermentation until brines reached a pH of 4.3 (4 months). The samples were coded as Az (Azzeradj) and Sig (Sigoise), followed by T₀ (before processing or raw olives), T₁ (after lye treatment and washing), T₂ (after fermentation: fermented fruits), respectively, to identify the successive processing phases. Therefore, the final acronyms for samples were: AzT₀, AzT₁, AzT₂, SigT₀, SigT₁, and SigT₂.

2.2 Analyses on fruit olives

Physical characteristics (Weight, width, length) of pulp and stone were performed by a digital caliper (150 mm (6")). Moisture (Tovar, Romero, Girona, & Motilva, 2002), oil content [Commission Regulation (EEC) No 2568/91 of 11 July 1991)], flavonoids (Djeridane et al., 2006) and total phenols (Favati, Caporale, & Bertuccioli, 1994) of olives were determined.

2.3. Oil extraction

Cold extraction of the oil was carried out by a laboratory mill (Levi-Dilon-Lerogsame) which consists of three basic elements: a hammer crusher, thermo-beater (mixer) and a pulp centrifuge. 3 kg of each sample was ground by a crusher, kneaded for 45 min and then submitted to vertical centrifugation (4845 rpm during 2 min) to extract the oil. The oil was transferred into dark glass bottles and stored at 4°C until analysis.

2.4 Analysis of lipid (oil) fraction

2.4.1. Oil quality index

Acidity, peroxide value (PV) and UV spectrophotometric indexes (measured at 232 and 270 nm) were determined according to the analytical methods described by the European Union regulation (Commission Delegated Regulation (EU) 2016/2095 of 26 September 2016 amending Regulation (EEC) No 2568/91)

2.4.2. Carbonylic compounds

Two volatiles compounds (Hexanal and Nonanal) were derivatized with 2,4 dinitrophenylhydrazine and determined by HPLC system. Four hundred milligrams of olive oil and 100 µL of internal standard solution in hexane (0.5 mg dodecanal/mL of hexane) were mixed and 1 mL of 2, 4 dinitrophenylhydrazine (0.1 % in acetonitrile 0.01N (HClO₄)) was added. The mixture was left to react in ultrasonic bath for 15 min at least. Then the solution was centrifuged at 5000 rpm for 15 min. The acetonitrile phase (5 µL) was injected in HPLC system constituted by a quaternary gradient pump P4000 (ThermoFinnigan, USA) and spectrophotometric detector UV6000LP (ThermoFinnigan). A reverse phase C18 Spherisorb

ODS2 3 μm , $l=25\text{ cm}$, i.d. = 4.0 mm (Chrom, Germany) was used. The mobile phase was constituted by 45 % water (A), 20 % acetonitrile (B), 35 % methanol (C) with a linear gradient for 60 minutes to 0 %A, 50 %B, and 50 % C. The flow rate was 1 mL/min. The hexanal and nonanal were quantified by measuring the peak area recorded at 360 nm and expressed as dodecanal in mg/kg of oil (Rovellini, & Cortesi, 2002).

2.4.3. Fatty acids compounds

The methyl ester derivatives of fatty acid composition were prepared with potassium hydroxide according to methods described in **EEC Regulation (Commission regulation (ECC) 1991) and Commission Implementing Regulation (EU) 2015)** and analyzed by 7890 Agilent gas chromatography (Agilent, Germany) equipped with a FID detector and a split/split less injector. A capillary column HP88 Agilent 112-88177 (Germany) (100 m x 0.25 mm, 0.20 μm) was used. The injector and detector temperatures were 260 °C and 280 °C respectively. The oven temperature was 1 min at 60 °C, from 60 °C to 165 °C at 10 °C/min, 1min at 165 °C, from 165 °C to 225 at 2 °C/min, 25 min at 225 °C. Helium was used as carrier gas. Fatty acids were identified by comparing their retention times with those of standard compounds. Results were expressed in percentages of the total fatty acids.

2.4.4. Tocopherols

An HPLC (Thermo Finnigan, USA) connected to a photodiode array detector was used to determine tocopherols composition. A reversed-phase silica column Allsphere ODS2 (5 μm , 250 mm $\times$ 4.6 mm; Alltech) (Grace, Belgium) was used. The mobile phase (acetonitrile: methanol, 1:1) was delivered to the column at a flow rate of 1.3 mL/min. The eluate was monitored at 292 nm. The different isomeric forms were identified comparing other vegetable oils typical for their tocopherol content distribution. The quantification was conducted utilizing an external calibration solution of alpha-tocopherol in acetone (0.01 mg/mL) (Rovellini, Azzolini, & Cortesi, 1997).

2.4.5. Total phenolic compounds

The extraction of polyphenols from oil was performed with methanol: water (80:20) (Ollivier et al. (2004), the total phenolic content was determined using the Folin–Ciocalteu reagent (Favati et al. 1995). The absorbance was read at 765 nm and the total phenol content was expressed as mg equivalent of gallic acid per kilogram of oil (mg GAE/kg) in reference to calibration curve ($y=5.076x+0.01$, $R=0.993$).

2.4.6. Analysis of individual phenolic compounds

Oil polyphenols were extracted with methanol: water (80:20 v/v) using an ultrasonic bath for 15 min and centrifuged at 5000 rpm for 25 minutes (IOC / T.20 Doc N.29) (2017). An internal standard (1 mL of 0.015 mg/ml of syringic acid in methanol: water (80:20 v/v) was added to the olive oil. After filtering the methanolic phase through a 0.45 µm filter, an aliquot was injected into the HPLC (ThermoFinnigan, USA), equipped with a UV detector monitored at 280 nm and 240 nm. A Spherisorb ODS-2 reversed column Alltima (5mm, 250 mm, id. 4.6 mm; Grace Belgium) was used. The separation was achieved with an elution gradient (Rovellini, Cortesi, & Zaganelli, 2010) having an initial composition of 96 % acidified water (water and *o*-phosphoric acid (99.8:0.2 v/v), 2 % methanol and 2 % acetonitrile. A flow rate of 1 ml/min was used in all the runnings. The main phenolic compounds (natural and oxidized form) were identified in comparison with relative retention times and UV spectra of standards and expressed as (mg/kg).

2.4.7. Antioxidant Activity

2.4.7.1. Radical scavenging activity (RSA) of oil against DPPH radical

The procedure reported by Ramadan, (2013) was used. Briefly, 3.9 ml of fresh DPPH solution 0.1mM was mixed with 1ml of diluted oil in toluene. The mixture was vortexed for 20 s at ambient temperature and absorbance was measured at 515 nm after 60 min of incubation. Inhibition percent was calculated from the following equation:

% inhibition= [(absorbance of control - absorbance of sample)/absorbance of control] x100.

The antioxidant capacity was also expressed as IC₅₀, IC₅₀ value is the concentration of the sample required to inhibit 50 % of radical of DPPH

2.4.7.2 Radical scavenging activity (RSA) of methanolic extracts against DPPH radical

The radical-scavenging activity of the methanolic extracts of the oil fraction against DPPH was determined (Amro, Aburjai, & Al-Khalil, 2002). The methanolic extract of oil was obtained using methanol: water (80:20) as described by Ollivier et al. (2004). An aliquot of 2 ml of DPPH solution (0.1 mM in methanol) was added to 2 mL of the methanolic extract. After 30 min incubation in the dark, the absorbance was measured at 515 nm. The result of radical scavenging activity was expressed as the inhibition percentage using the formula previously described.

2.8. Statistical analysis

All the results are reported as the mean values (n = 3) and were subjected to the analysis of variance using the Statistica 5.0 software package (StatSoft'97 edition) using the least significant difference (Newman–Keuls) test. Significance was defined at (p<0.05). The principal component analysis (PCA) and hierarchical ascending classification (HAC) were performed by XLSTAT 2009.

3. Results and Discussion

3.1. Characteristics and composition of olive fruits

Physical characteristics, phenolic and lipid content of fruits are reported in Table1. The fruits of both cultivars showed a similar weight and pulp /stone ratio. The flesh–to–pit ratio was 6.33 and 7.13 for Azzeradj and Sigoise, respectively. These physical characteristics confer a good attribute towards their elaboration as table olives. Processing induced an increase in olives humidity due to the osmosis phenomenon. Water content in the processed olives was

generally higher for olives treated with NaOH than in natural-processed (**Lombardi, Macciola, Iorizzo, & De Leonardis, 2018**).

Sigoise cv. is the most used for table olive processing in Algeria, and showed minor lipid content (30.55 %/DW) than the Azzeradj (41.06 %/DW), which has a dual destination (oil mill and table olives processing). Only the lipid fraction of Azzeradj variety recorded a significant decrease during the processing of table olives. The reduction of the oil content could be explained by the lipolytic action of the lipases of the fruits themselves or by the microorganisms present in the brine solution (López-López et al., 2009).

The pulp of Sigoise olives is characterized by a higher content of total phenols and flavonoids than Azzeradj ones and a noticeable decrease was exhibited after processing. Losses in total phenolic compounds after processing were 79.25 and 67.58 % for Azzeradj and Sigoise, respectively. Also, similar losses were recorded for the flavonoids. Treatment with sodium hydroxide followed by washing is more detrimental than fermentation; the total phenols losses were 71.40 and 56.55 % for Azzeradj and Sigoise, respectively. Lye-treatment hydrolyzes the phenolic compounds, also increases the permeability of cell membranes and the diffusion is higher and faster. Structural changes were found to be specific to each cultivar (**Bianchi, 2003**).

Similar results have been previously noticed for the same cultivars (**Mettouchi et al.2016**). These results corroborate those found for the Spanish varieties Manzanilla and Hojiblanca processed according to the Spanish style; the concentration of polyphenols in olive after processing was estimated at 20-30 % of the initial content (**Romero et al., 2004a**). Compared to other elaborations, Spanish-style green olives had the lowest phenolic content (**Romero et al., 2004a; Sahan, Cansev, & Gulen, 2013**).The losses of polyphenols are higher in those table olives elaboration process in which a NaOH solution is applied than the fermentation step (**Boskou, 2017**).

3.2. Lipid fraction (oil)

3.2.1. Oil quality indices

The oil quality indexes of the two olive cultivars were shown in Table 2. All the values were lower than the limits set by **EU Regulation (1991)** for the extra virgin olive oil category.

The acidity which represents the percentage of free fatty acids showed a slight decrease after alkaline treatment and an increase after the fermentation. Free fatty acids production could be catalyzed by fruits lipase or by those synthesized by the environmental microflora **(Ciafardini, &Gomes, 1995)**.

Significant decreases were noticed for peroxides index and K_{270} of both cultivars during processing. The lye-treatment could not only destroy the peroxides but also the secondary products resulting from their decomposition. The obtained values are in agreement with European standards (**Commission regulation (ECC) No 2568/91 of July (1991)**) for oils belonging to the extra-virgin category. Our results for oil quality indices are close to those obtained by **Lopez-Lopez et al. (2015)** for Spanish varieties (Manzanilla and Hojiblanca), but lower compared to an olive oil obtained from directly brined table olives (**Pasqualone et al., 2014**) and those reported for the Californian-style (**Lopez-Lopez et al., 2009a**). With regard to acidity, Lopez-Lopez et al. (2015) recorded low values for Manzanilla (0.25-0.56) and Hojiblanca (0.17-0.79) varieties prepared according to the Spanish style, while high values were noted for the Californian style (0.7- 1.2). Also, Pasqualone et al. (2014) found acidity values between 2.04 and 2.25 for directly brined table olives after 8 months of fermentation.

3.2.2. Hexanal and nonanal

3.2.2. Hexanal and nonanal

The flavor of table olives is related to the quality and quantity of volatile and non-volatile compounds. The volatile compounds in table olives are produced during fermentation by the action of endogenous enzymes (lipoxygenases) and exogenous enzymes (produced by lactic

bacteria, yeast, etc.) on major fruit constituents (**Sabatini, & Marsilio, 2008**). The two volatile compounds, hexanal and nonanal, belonging to the aldehyde fraction were analyzed (Table 2). Hexanal produced mainly by the LOX pathway was known to have a positive effect on aroma (green and sweet odors), while Nonanal, formed through the oxidation was associated with sensory defects (fatty, waxy, painty) (**Morales, Rios, & Aparicio, 1997**). The ratio hexanal/nonanal was used as an oxidation status of olive oils. The nonanal was found at low concentrations in olive oil (**Morales, Rios, & Aparicio, 1997**) and raw olives (de Castro, Higinio Sánchez, Cortés-Delgado, López-López, Montaña, 2019).

Significant increases in the order of 100 % were noted for hexanal. While for nonanal, an opposite trend was observed for both varieties. The reduction can be considered as a good indicator of the degradation of this volatile compound.

Recently, the effect of alkaline treatment and fermentation on the volatile composition of green olives was studied. Nonanal and hexanal and the major volatiles detected in fresh olives Manzanilla green olives decreased after alkaline treatment and after spontaneous fermentation. Around 60 % of the compounds identified in the fermented product were entirely produced during the fermentation step presumably originated through microbial action (**De Castro, Sánchez, Cortés-Delgado, López-López, & Montaña, 2019**).

3.2.3. Fatty acids

The results of fatty acid composition (Table 3) showed that the most abundant fatty acids were oleic, linoleic and palmitic acids. Stearic, palmitoleic and linolenic acids were present at low concentration. Azzeradj cultivar was characterized by the highest content on oleic acid (75.37 %) and linoleic acid (11.96 %) than Sigoise cultivar (70.81 and 6.25 %, respectively).

Both cultivars have similar contents of palmitic acid. Sigoise variety showed slightly higher content on C18:3 than the European standard for extra-virgin olive oil (EVOO). Similar results were noticed for the Spanish Hojiblanca cultivar (**Lopez-Lopez et al; 2015**) and Meksi

Tunisian cultivar (Issaoui et al., 2011). All the other fatty acids were below the limit established in the EU Regulation for EVOO (Commission Implementing Regulation (EU) No 1348/2013).

The processing of the table olives of both cultivars caused a little variation on the content of C18:2 and C18:3 which reflected a slight sensitivity of these fatty acids. Conversely, saturated and monounsaturated fatty acids remained almost stable. The presence of large amounts of monounsaturated fatty acids (MUFAs) and their stability is important for the nutritional value and the positive effects on human health.

The trans fatty acids, including trans-linoleic acid (C18: 2t) and trans-linolenic acid (C18: 3t) were absolutely absent, while trans-oleic acid (C18: 1t) showed a low value of 0.04% which was below the limits required by the Commission Regulation (EU); ≤ 0.05 (Commission Implementing Regulation (EU) 2013).

Principal component analysis (PCA) was applied to evaluate the variability of fatty acids between oil samples according to the cultivar and the steps of processing. As shown in Fig.1 (A), PCA analysis indicated that two factors account for 92.65% of the total variance (F1: 85.11 % and F2: 7.64%). Based on Factor 1, the PCA has segregated clearly the samples from Azzeradj (on the left) and Sigoise (on the right). The variables that most contributed to the differentiation among cultivars were those strongly correlated (positively or negatively) with Factor 1. Azzeradj was rich in C18:1, C18:0, MUFA, SFA, C17:0; C17:1, and C20:0, while Sigoise is rich in C18:2, C18:3n-3, PUFA, C20:1, and C16:1.

The first factor was correlated positively with C18:1, C18:0; C17:0; C17:1 C20:0, C22:0, MUFA, SFA. The clear individualization of samples according to the variety was the result of genetic variability in relation to the parameters analyzed. Segregation by treatments within cultivars was achieved by Factor 2. The variable that contributed to this discrimination was only 16:0 (Fig. 1A). The transformation had no significant effect on fatty acids of the same

variety. There is no separation between the fresh sample and the other treated samples. The results obtained (Fig. 1B) reveal a classification of individuals into 2 groups: Group 1 composed of samples belonging to cultivar Azzeradj, and the second group represented by samples of Sigoise. Globally, our results are in agreement with those obtained by **Lopez-lopez et al. (2015)** who noted good stability of fatty acids of Manzanilla and Hojiblanca olives processed as green Spanish-style. Also, **Issaoui et al. (2011)** stated that the differences observed for fatty acids of the Meski, Picholine and Manzanilla olive cultivars elaborated according to two traditional Tunisian processes were related to the variety and not to the treatment.

3.2.4. Tocopherols

Tocopherols are lipophilic compounds with nutritional properties and antioxidant activity against free radicals by protecting polyunsaturated fatty acids (**Sakouhi et al., 2008**). Results in table 4 showed a predominance of α -tocopherol (>95 %) in the composition of both cultivars, while γ , β , and δ -tocopherols were present at very low levels. Sigoise variety showed higher concentration in total tocopherols (176.99 mg/kg) than the Azzeradj (131.03 mg/kg). The α -tocopherol content depends on the cultivar and decreased by 32.73 % and 36.18 % for Sigoise and Azzeradj, respectively. However, the most important losses were related to the step of fermentation. Despite the losses recorded, the residual contents were higher than the values reported for 30 samples of commercial Italian olives which ranged between 25 and 90 mg/ kg (**Sagratini et al., 2013**). Contradictory results have previously been found for the effect of processing on the tocopherol composition of green Halkidiki and Conservolea olives (**Hassapidou, Balatsouras, & Manoukas, 1994**) and Tunisian autochthonous cultivars (Sakouchi et al. 2008). The tocopherol composition of alkali-treated green Manzanilla's olives was not affected by processing (Montaño, Casado, De Castro, Sánchez, & Rejano, 2005). According to **Nikzad, Sahari, Vanak, Safafar, Boland-nazar**

(2013) who studied 5 olive cultivars, the major damage occurred in lye step (Zard, Fishomi, Ascolana, Amigdalolia, and Conservolea).

3.2.5. Individual Phenolic compounds

The main phenolic compounds identified by HPLC were reported in table 5. The secoiridoid polyphenols (oleuropein, ligustroside, and their derivatives) represented the major fraction for both varieties. Sigoise cv. contained high amounts of oleuropein derivatives but less ligustroside derivatives and lignans than Azzeradj cv. Luteolin, apigenin, oleocanthal, phenolic acids and alcohols were present at lower concentrations.

As seen in Table 5, the main losses were noticed after the lye treatment-washing step. The alkaline solution produces the breakage of the ester bond of oleuropein molecule with the consequent formation of non-bitter compounds (Brenes & de Castro, 1998). Also, the permeability of the olive membranes is affected by the alkali treatment, facilitating a quick diffusion of nutrients between fruit and brine. Oleuropein and hydrophilic components move from olive fruit to the brine, while salt (NaCl) does the opposite. The presence of antimicrobial compounds in olive brines has been well reported, but an intense lye-treatment (2/3 lye depth penetration of flesh olives) avoids the formation of inhibitors of lactic acid bacteria allowing an adequate fermentation (Medina et al., 2008). The endogenous enzymes present in the olive fruit (β -glucosidase and esterase), responsible for debittering in not treated with alkali table olives, were degraded or inactivated during the lye-treatment (Pandey & Ramachandran, 2008), so most of the hydrolysis of phenolic compounds is due to the alkaline conditions. Also, the hydrolysis of elenolic acid-glucoside to glucose and elenolic acid continued during the fermentation step by the acid conditions (Ramirez et al. 2016), or by the enzymatic action of some lactic acid bacteria (Ramirez et al., 2017).

Moreover, the oxidation of phenolic compounds is an inevitable phenomenon and increases significantly during processing (Table 5). The ratio oxidized biophenols/total phenols were from 5.63 to 21.06 for Azzeradj and from 3.20 to 18.42 for Sigoise.

Principal Component Analysis (PCA) was applied to evaluate the variability of phenolic compounds according to the cultivar and the steps of Spanish-style table olive processing. As shown in Figure 2 A, two factors accounted for 90.77 % of the total variance (F1: 73.71 %, F2: 17.06 %). The first factor was correlated positively with total phenols, oleuropein derivatives, ligustroside derivatives, oleocanthal, lignans, lutein, apigenin, phenolic acids, hydroxytyrosol, decarboxymethylelenolic acid, total secoiridoid acids and elenolic acid. The second axis was positively correlated with total alcohols, oleuropein and tyrosol. Grouping raw olives of both cultivars in the same group but in two different subgroups indicated that the qualitative and quantitative composition of the phenolic compounds showed some variability. The influence of the elaboration process on the phenolic compounds was clearly shown by another opposed group to the first (right). The segregation of the samples into two groups was essentially based on their different phenolic contents. Within the same cultivar, raw and processing samples (lye treatment-washing and fermentation) were opposed, showing important degradation of phenolic compounds due to the processing of the different cultivars as shown by clustering analysis (figure 2 B). The processing samples were positively correlated with hydrolysis, oxidation ratio and oxidized phenols, and negatively correlated with all phenolic compounds.

The phenolic profile of the oil phase of green table olives processed according to the Spanish-style has been studied by **Romero et al. (2004a)**. Oleuropein and ligustroside aglycons were the predominant compounds in the oil fraction of raw fruit, but not in the lipidic fraction of processed fruit, suggesting the hydrolysis of aglycons during alkaline and fermentative process. On the contrary, hydroxytyrosol, tyrosol and vanillic acid were detected in both. The

evolution of the phenolic compounds of the oil phase during the fermentation of natural black olives was also studied (Romero, Brenes, García, Garcia, & Garrido, 2004b). Phenolic compounds were quickly hydrolyzed during the fermentation process and disappeared after three months of processing. Consequently, the concentrations of their hydrolysis products increased with time.

3.2.6. Antioxidant activity

DPPH is a commonly used substrate (free radical) for fast and easy evaluation of the antioxidant activity due to its stability, reliability and the simplicity of the assay. Table 6 showed the antioxidant capacity of oil fraction and its methanolic extracts against radical DPPH. Results indicated that the methanolic extracts of the raw sample of Azzeradj exhibited a high antioxidant potential (95.66 %) than Sigoise (80.79 %). Processing induced an important decrease in antioxidant capacity for the two cultivars, from 95.6 to 37.8 % for Azzeradj and from 80.79 to 16.62 % for Sigoise. A significant correlation was noticed between antioxidant activity and phenolic compounds, particularly for total phenols ($r=0.72$), oleuropein ($r=0.62$) and ligustroside derivatives ($r=0.84$). Previous studies showed that the antioxidant capacity of table olives is probably related to polyphenol content, including hydroxytyrosol and tyrosol (Baiano, Gambacorta, Terracone, Previtali, & La Notte, 2009; Mettouchi et al., 2016).

The oil of the raw olives of Sigoise cv. recorded high antiradical capacity (89.65 %) than Azzeradj (51.53 %). This trend is due to the content on tocopherols. Indeed, Sigoise showed higher total and alpha tocopherols contents (176.99 and 168.55 mg/kg) than Azzeradj (131.03 and 128.50 mg/kg).

The IC_{50} registered for Sigoise (17.99 mg/ml) was lower than Azzeradj (32.29 mg/ml). It recorded very important increases during processing, indicating a significant decrease in antioxidant activity for both cultivars.

The carotenoids can also contribute to the antioxidant activity of the oil fraction (data not shown). The alkaline treatment of olive fruits does not produce any change in the carotenoid pigments since they are alkali-stable compounds (**Ramírez, Gandul-Rojas, Romero, Brenes, & Gallardo-Guerrero, 2015**).

Globally, the results showed that antioxidant capacity (oil and methanolic extract) of table olives from the Azzeradj and Sigoise varieties decreased with the loss of phenolic compounds and tocopherols. Similar results were obtained for Tunisian natural table olives by **Issaoui et al., (2011)** that showed a strong positive correlation between the total equivalent antioxidant capacity and total phenolic content. Usually, green Spanish-style olives showed the lowest antioxidant activities in comparison with other table olives elaborations (**Sahan et al., 2013**).

4. Conclusion

The study revealed the effects of the main steps of Spanish-style green olive processing (alkaline treatment and fermentation) on the quality, compounds and antioxidant activity of lipid fraction. The quality parameters (acidity, PV, K_{232} , K_{270}) registered slight variations; they were always below the limit established for EVOO. Processing did not cause any effect on fatty acids. Substantial losses were found for total and individual phenolic compounds during the processing. The most significant change occurred at the lye treatment rather than the fermentation. Tocopherols, mainly the α -isomer, are also affected, especially after the lye treatment and washing. As a consequence, an important reduction in the antioxidant value was noticed. Future studies will be necessary to improve the processing methods and develop new techniques, which preserve phenolic compounds and fat quality due to its important role in human health.

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601 Figure captions.

602 Figure 1. Def. Title Representation of factorial plans 1–2 of the principal component analysis
603 (A) and Hierarchical Clustering Analysis (B) as a function of fatty acids

604 Abbreviation: SFA (Saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA
605 (polyunsaturated fatty acids).

606 The acronyms for samples are: AzT₀, AzT₁, AzT₂, SigT₀, SigT₁, and SigT₂.

607 Az (*Azzeradj*); Sig (*Sigoise*); T₀ (raw olives); T₁ (treatment lye- washing); T₂ (fermentation).

608

609 Figure 2. Def. Title Representation of factorial plans 1–2 of the Principal Component
610 Analysis (A) and Hierarchical Clustering Analysis (B) as a function of phenolic contents:

611 hydroxytyrosol (HyT); tyrosol (Tyr); total alcohols (Tot. Alc); oleuropein (Ole); oleuropein

612 derivatives (Der–Ole); ligustrosidederivatives (Der-Lig);oleocanthal (Oleoc); phenolic acids

613 (Tot.Phe-Acid);total flavonoids (Tot.Flav);luteolin (Lut); apigenin (Apig);oxidised total

614 biophenols (Oxyd.phe); oxidised ratio (Oxid.Ratio): (Oxidised biophenols/ total biophenols

615 (%)) hydrolysis ratio (Hydro. Ratio)(total aromatics alcohols/ total biophenols) (%); total

616 secoiridoidic acids (Sec. Acid); decarboxymethylelenolic acid (Dec.met.ele.acid); elenolic

617 acid (Ele.Acid); total phenols (Tot.Phe).

618 The acronyms for samples are: AzT₀, AzT₁, AzT₂, SigT₀, SigT₁, and SigT₂.

619 Az (*Azzeradj*); Sig (*Sigoise*); T₀ (raw olives); T₁ (treatment lye- washing); T₂ (fermentation).

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Table 1 Physicachemical parameters of Spanish-style green table olives

Samples	Azzeradj cv.			Sigoise cv.		
	Az T ₀	Az T ₁	Az T ₂	Sig T ₀	Sig T ₁	Sig T ₂
Fruit weight	4.18 ±0.83	4.15± 0.93	4.61± 0.89	4.28±0.41	4.06 ±0.45	4.08±0.57
Moisture (%)	49.97 ± 1.80 ^a	56.36 ± 3.85 ^b	59.72 ± 2.51 ^{bc}	59.38 ± 3.65 ^{bc}	66.35 ± 1.84 ^{bc}	65.89 ± 1.79 ^c
Neutral total fat (% of	41.06 ± 0.39 ^b	40.87 ± 0.49 ^b	38.72 ± 0.73 ^b	30.55 ± 0.95 ^a	30.68 ± 0.11 ^a	28.56 ± 0.70 ^a
Total polyphenols (mg/Kg)	19456,92 ± 119,82 ^d	5019,90 ± 188,82 ^b	3988,67 ± 59,09 ^a	15830, 87 ± 50,00 ^e	6877 , 96 ± 206,73 ^c	5131,38 ± 208 ^b
Total flavonoids meq	820.73 ± 2.86 ^e	277.51 ± 1.89 ^c	204.92 ± 2.14 ^a	1061.20 ± 3.27 ^f	329.89 ± 3.27 ^d	234.20 ± 071 ^b

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Table1. Physicachemical parameters of Spanish-style green table olives

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Means in the row followed by different letters are significantly different (p<0.05). Abbreviations: Az (*Azzeradj*)

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Sig (*Sigoise*), T₀ (raw olives), T₁ (lye treatment - washing), T₂ (fermentation).

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Table 2. Quality indexes of lipid fraction of Spanish-style green table olives

	Azzeradj			Sigoise		
	AzT ₀	AzT ₁	AzT ₂	SigT ₀	SigT ₁	SigT ₂
Acidity (%)	0.32 ± 0.08 ^b	0.22 ± 0.08 ^b	0.27 ± 0.00 ^{ab}	0.18 ± 0.08 ^{ab}	0.13 ± 0.00 ^a	0.22 ± 0.08 ^{ab}
Peroxide index	9.83 ± 1.26 ^b	6.00 ± 0.87 ^a	5.00 ± 1.32 ^a	9.00 ± 0.50 ^b	13.83 ± 0.29 ^c	13.67 ± 0.29 ^c
K ₂₃₂	1,681±0.003	1,30±0.007 ^b	1,24±0.006 ^a	1,78±0.003 ^d	2,04±0.01 ^e	2,09±0.005 ^f
K ₂₇₀	0,105±0.002	0,082±0.001 ^d	0,036±0.001 ^a	0,10±0.00 ^c	0,070±0.002 ^b	0,072±0.00 ^b
Hexanal	45.98 ± 4.21 ^a	47.66 ± 0.47 ^a	91.69 ± 2.57 ^b	87.76 ± 2.40 ^b	154.35 ± 2.11 ^c	177.36 ± 6.97 ^d
Nonanal	47.87 ± 4.21 ^b	32.61 ± 1.24 ^a	26.29 ± 0.74 ^a	42.60 ± 0.42 ^b	45.27 ± 2.86 ^b	73.32 ± 3.77 ^c
Total	90.34 ± 2.66 ^a	80.27 ± 0.77 ^a	117.98 ± 2.20 ^b	130.36 ± 1.08 ^c	199.61 ± 0.75 ^d	250.67 ± 10.72 ^e

Means in the row followed by different letters are significantly different (p<0.05). Abbreviations: Az (Azzeradj) Sig (Sigoise), T₀ (raw olives), T₁ (treatment lye- washing), T₂ (fermentation),

Table 3: Fatty acids composition of Azerradj and Sigoise Spanish-style green table olives (expressed as %).

	Azzeradj			Sigoise		
	AzT ₀	AzT ₁	AzT ₂	SigT ₀	SigT ₁	SigT ₂
C14:0	0.01 ± 0.0 ^a	0.01 ± 0.0 ^a	0.01 ± 0.0 ^a	0.02 ± 0.01 ^b	0.03 ± 0.0 ^b	0.03 ± 0.0 ^b
C15:0	0.01 ± 0.0	0.00 ± 0.0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.0	0.01 ± 0.0
C16:0	12.33 ± 0.06	13.01 ± 0.61	13.20 ± 0.12	12.64 ± 0.03	13.13 ± 0.18	12.52 ± 0.16
C16:1	0.76 ± 0.01 ^a	0.76 ± 0.04 ^a	0.76 ± 0.01 ^a	0.97 ± 0.01 ^{bc}	1.01 ± 0.01 ^c	0.93 ± 0.01 ^b
C17:0	0.18 ± 0.0 ^b	0.19 ± 0.01 ^b	0.20 ± 0.0 ^c	0.04 ± 0.0 ^a	0.04 ± 0.0 ^a	0.04 ± 0.0 ^a
C17:1	0.25 ± 0.0 ^b	0.26 ± 0.01 ^c	0.27 ± 0.0 ^c	0.06 ± 0.0 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a
C18:0	3.43 ± 0.01 ^b	3.42 ± 0.08 ^b	3.54 ± 0.01 ^c	1.63 ± 0.01 ^a	1.60 ± 0.01 ^a	1.66 ± 0.01 ^a
C18:1	75.37 ± 0.05 ^c	75.02 ± 0.05 ^c	74.87 ± 0.12 ^c	70.82 ± 0.08 ^{ab}	70.55 ± 0.25 ^a	71.30 ± 0.13 ^b
C18:2	6.25 ± 0.01 ^c	6.00 ± 0.01 ^b	5.79 ± 0.01 ^a	11.96 ± 0.04 ^f	11.80 ± 0.04 ^e	11.69 ± 0.01 ^d
C18:3	0.47 ± 0.0 ^b	0.44 ± 0.0 ^a	0.44 ± 0.0 ^a	1.13 ± 0.01 ^e	1.12 ± 0.0 ^d	1.09 ± 0.01 ^c
C20:0	0.49 ± 0.01 ^b	0.48 ± 0.04 ^b	0.49 ± 0.01 ^b	0.26 ± 0.01 ^a	0.25 ± 0.0 ^a	0.26 ± 0.01 ^a
C20:1	0.26 ± 0.00 ^a	0.25 ± 0.02 ^a	0.25 ± 0.01 ^a	0.32 ± 0.00 ^b	0.30 ± 0.01 ^b	0.32 ± 0.01 ^b
C22:0	0.12 ± 0.00 ^b	0.11 ± 0.01 ^b	0.11 ± 0.0 ^b	0.06 ± 0.00 ^a	0.06 ± 0.00 ^a	0.06 ± 0.00 ^a
C24:0	0.05 ± 0.00	0.04 ± 0.01	0.05 ± 0.0	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
C24:1	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.01 ± 0.0 ^b	0.01 ± 0.0 ^b	0.02 ± 0.01 ^b	0.01 ± 0.0 ^{ab}
trans C18:1	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
trans C18:2	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.0	0.02 ± 0.00	0.02 ± 0.00
trans C18:3	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.0	0.01 ± 0.01	0.01 ± 0.00
Amount trans	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.010	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
SFA	16.625 ± 0.00 ^b	17.27 ± 0.00 ^a	17.615 ± 0.00 ^a	14.72 ± 0.03 ^a	15.165 ± 0.00 ^a	14.63 ± 0.16 ^a
MUFA	76.65 ± 0.04 ^b	76.31 ± 0.00 ^b	76.155 ± 0.00 ^b	72.185 ± 0.00 ^a	71.935 ± 0.00 ^a	72.615 ± 0.00 ^a
PUFA	6.725 ± 0.01 ^c	6.44 ± 0.01 ^b	6.235 ± 0.01 ^a	13.10 ± 0.03 ^f	12.92 ± 0.04 ^e	12.77 ± 0.01 ^d
C18:1/C16:0 ratio	6.11 ± 0.03 ^b	5.78 ± 0.31 ^{ab}	5.67 ± 0.06 ^{ab}	5.60 ± 0.02 ^{ab}	5.37 ± 0.09 ^a	5.70 ± 0.08 ^{ab}

SFA: saturated fatty acids; MUFA: monounsaturated; PUFA: polyunsaturated fatty acids
Means in the row the column followed by different letters are significantly different (p<0.05). Az (*Azzeradj*), Sig (*Sigoise*), T₀ (raw olives), T₁ (treatment lye- washing), T₂ (fermentation).

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Table 4: Tocopherols composition of *Azzeradj* and *Sigoise* green table olives.

	Azzeradj			Sigoise		
	AzT ₀	AzT ₁	AzT ₂	SigT ₀	SigT ₁	SigT ₂
δ- Tocopherol	1.00 ± 0.00 ^b	1.00 ± 0.00 ^b	1.00 ± 0.00 ^b	0.54 ± 0.04 ^a	0.44 ± 0.09 ^a	0.45 ± 0.02 ^a
γ- Tocopherol	1.28 ± 0.23 ^a	1.17 ± 0.01 ^a	1.02 ± 0.21 ^a	5.57 ± 0.35 ^b	5.14 ± 0.32 ^b	5.00 ± 0.21 ^b
β- Tocopherol	1.26 ± 0.07 ^b	1.24 ± 0.07 ^b	0.84 ± 0.23 ^a	2.23 ± 0.04 ^c	2.45 ± 0.04 ^c	2.51 ± 0.17 ^c
α- Tocopherol	128.50 ± 2.30 ^c	128.74 ± 1.84 ^c	90.63 ± 2.45 ^a	168.65 ± 0.52 ^c	144.38 ± 1.54 ^d	107.63 ± 2.35 ^b
Total Tocopherol	131.03 ± 2.14 ^c	131.15 ± 1.75 ^c	92.48 ± 2.88 ^a	176.99 ± 0.18 ^c	152.40 ± 1.73 ^d	115.57 ± 2.71 ^b

Means in the row followed by different letters are significantly different (p<0.05). Az (*Azzeradj*) Sig (*Sigoise*), T₀ (raw olives), T₁ (treatment lye- washing), T₂ (fermentation),

Table 5: Polyphenols content of the lipid fraction of Azzeradj and Sigoise green table olives

	Azzeradj			Sigoise		
	AzT ₀	AzT ₁	AzT ₂	SigT ₀	SigT ₁	SigT ₂
Total Biophenols	276.02 ± 2.17 ^f	77.85 ± 2.14 ^a	62.22 ± 0.41 ^b	215.66 ± 2.14 ^e	66.62 ± 1.65 ^c	60.21 ± 0.32 ^a
Natural total Biophenols	260.51 ± 2.14 ^f	68.89 ± 2.14 ^d	48.34 ± 0.04 ^b	208.76 ± 2.14 ^e	57.24 ± 1.59 ^c	42.58 ± 0.4 ^a
Total aromatic Alcohols	9.00 ± 0.10 ^b	8.60 ± 0.57 ^{ab}	8.02 ± 0.05 ^a	14.76 ± 0.10 ^c	13.02 ± 0.06 ^d	10.66 ± 0.04 ^c
Hydroxytyrosol	2.41 ± 0.11 ^e	1.95 ± 0.07 ^d	0.06 ± 0.01 ^a	2.65 ± 0.03 ^f	0.58 ± 0.04 ^c	0.39 ± 0.01 ^b
Tyrosol	6.59 ± 0.01 ^a	7.35 ± 0.21 ^b	7.95 ± 0.05 ^c	12.11 ± 0.07 ^e	12.45 ± 0.02 ^f	10.27 ± 0.04 ^d
Oleuropein	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.08 ± 0.00 ^b	0.01 ± 0.00 ^a	0.01 ± 0.01 ^a
Oleuropein Derivatives	72.40 ± 0.75 ^d	14.66 ± 3.76 ^c	7.32 ± 0.33 ^b	96.18 ± 0.23 ^e	15.02 ± 1.39 ^c	9.60 ± 0.09 ^a
Ligustroside Derivatives	91.35 ± 0.6 ^e	25.40 ± 0.28 ^c	15.97 ± 0.06 ^a	62.69 ± 0.52 ^d	26.21 ± 0.04 ^c	19.81 ± 0.30 ^b
Oleocanthal	30.10 ± 0.20 ^d	1.80 ± 0.28 ^b	0.20 ± 0.02 ^a	23.94 ± 0.17 ^c	1.85 ± 0.07 ^b	0.33 ± 0.13 ^a
Total Lignane	71.10 ± 0.44 ^f	20.04 ± 0.04 ^c	20.87 ± 0.14 ^d	27.43 ± 0.10 ^e	14.99 ± 0.01 ^b	13.25 ± 0.01 ^a
Total phenolic Acids	17.45 ± 0.07 ^f	8.78 ± 0.11 ^d	5.19 ± 0.10 ^b	13.53 ± 0.08 ^e	1.02 ± 0.15 ^a	7.93 ± 0.18 ^c
Total Flavonoids	8.22 ± 0.02 ^b	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	8.94 ± 0.04 ^c	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
Luteolin	5.71 ± 0.08 ^b	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	6.97 ± 0.02 ^c	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
Apigenin	2.51 ± 0.10 ^c	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	1.97 ± 0.01 ^b	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
Oxidised total Biophenols	8.10 ± 0.21 ^b	8.97 ± 0.39 ^c	12.89 ± 0.37 ^d	6.89 ± 0.19 ^a	9.38 ± 0.06 ^c	9.62 ± 0.07 ^c
Oxidised ratio: (Oxidised biophenols/ total biophenols)	5.63 ± 0.02 ^b	11.50 ± 0.71 ^c	21.06 ± 0.46 ^f	3.20 ± 0.09 ^a	14.09 ± 0.26 ^d	18.42 ± 0.25 ^e
Hydrolysis ratio: (Total aromatics alcohols/ total	3.26 ± 0.04 ^a	3.43 ± 0.07 ^a	13.09 ± 0.17 ^d	6.85 ± 0.05 ^b	9.13 ± 0.28 ^c	20.42 ± 0.21 ^e
Total secoiridoidic acids	199.00 ± 2.14 ^d	10.50 ± 2.14 ^b	1.97 ± 0.03 ^a	32.51 ± 0.04 ^c	10.20 ± 0.99 ^b	2.35 ± 0.14 ^a
Decarboxymethyl elenolic acid	2.00 ± 0.01 ^e	1.00 ± 0 ^c	0.09 ± 0.01 ^a	1.73 ± 0.02 ^d	0.85 ± 0.07 ^b	0.08 ± 0.00 ^a
Elenolic acid	197.00 ± 2.14 ^d	9.50 ± 3.54 ^b	1.89 ± 0.04 ^a	30.79 ± 0.02 ^c	9.35 ± 0.92 ^b	2.27 ± 0.14 ^a
Total oxidised decarboxy-methyl- elenolic acid	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.03 ± 0.01 ^b	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a

Means in the row followed by different letters are significantly different (p<0.05). Az (*Azzeradj*) Sig (*Sigoise*), T₀ (raw olives), T₁ (treatment lye- washing), T₂ (fermentation).

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745 **Table 6:** Antioxidant activity of lipid fraction of Azerradj and Sigoise green table olives.

	Azzeradj			Sigoise		
	AzT ₀	AzT ₁	AzT ₂	SigT ₀	SigT ₁	SigT ₂
Total polyphenols (mg GAE/kg oil)	338.32 ± 0.00 ^e	54.92 ± 2.46 ^a	34.37 ± 1.23 ^b	247.32 ± 0.68 ^d	47.97 ± 1.22 ^c	33.78 ± 5.40 ^b
Percentage (%) of inhibition of the methanolic extracts	95.66 ± 0.31 ^f	44.30 ± 0.62 ^a	37.80 ± 0.71 ^d	80.79 ± 0.15 ^e	24.22 ± 0.62 ^c	16.62 ± 0.27 ^b
Percentage (%) of inhibition of the oil	51.53 ± 0.10 ^e	27.92 ± 0.54 ^a	20.53 ± 0.44 ^b	89.65 ± 1.42 ^f	53.00 ± 1.42 ^d	32.78 ± 0.39 ^c
EC ₅₀ (mg/ml)	32.29 ± 2.1 ^b	370 ± 10.5 ^d	567.00 ± 21.6 ^f	17.99 ± 2.3 ^a	296 ± 7.50 ^c	414 ± 12.0 ^e

746 Means in the row the column followed by different letters are significantly different (p<0.05).

747 Az (*Azzeradj*), Sig (*Sigoise*), T₀ (raw olives), T₁ (treatment lye- washing), T₂ (fermentation).

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Figure 1. Representation of factorial plans 1–2 of the principal component analysis (A) and Hierarchical Clustering Analysis (B) as a function of fatty acids
Abbreviation: SFA (Saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids). Az (*Azzeradj*); Sig (*Sigoise*); T₀ (raw olives); T₁ (treatment lye- washing); T₂ (fermentation).

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Fig. 2 Representation of factorial plans 1–2 of the Principal Component Analysis (A) and Hierarchical Clustering Aanalysis (B) as a function of phenolic contents: hydroxytyrosol (**HyT**); tyrosol (**Tyr**); total alcohols (**Tot. Alc**); oleuropein (**Ole**); oleuropein derivatives (**Der–Ole**); ligustroside derivatives (**Der-Lig**); oleocantal (**Oleoc**); phenolic acids (**Tot.Phe-Acid**); total flavonoids (**Tot.Flav**); luteolin (**Lut**); apigenin (**Apig**); oxidised total biophenols (**Oxyd.phe**); oxidised ratio (**Oxid.Ratio**): (Oxidised biophenols/ total biophenols (%) hydrolysis ratio (**Hydro. Ratio**) (total aromatics alcohols/ total biophenols) (%); total secoiridoidic acids (**Sec. Acid**); decarboxymethyl elenolic acid (**Dec.met.ele.acid**); elenolic acid (**Ele.Acid**); total phenols (**Tot.Phe**).

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The samples were coded as Az (*Azzeradj*) and Sig (*Sigoise*), followed by T₀ (before processing or raw olives), T₁ (after treatment with lye and washing), T₂ (after fermentation), The acronyms for samples are: AzT₀, AzT₁, AzT₂, SigT₀, SigT₁, and SigT₂.

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