

Chemical characterisation and antioxidant activity of Spanish-style green olives of the Azerradj and Sigoise cultivars

Hocine KERKOUR¹
Abderezak TAMENDJARI^{1*}
Eduardo MEDINA²
Fadila AIT CHABANE¹
Pierangela ROVELLINI³
Paola FUSARI³

¹ Laboratoire de Biochimie Appliquée
Faculté des sciences de la nature
et de la vie - Université de Bejaia Algeria
Bejaia, Algeria

² Food Biotechnology Department
Instituto de la Grasa, IG-CSIC
Seville, Spain

³ INNOVHUB-SSI
Area Oli e Grassi
Via Giuseppe Colombo, 79
20133 Milano, Italy

CORRESPONDING AUTHOR:
E-mail: abderezakt@yahoo.fr

Received: April 22, 2020
Accepted: July 16, 2020

The chemical composition and the antioxidant activity of two Algerian olive cultivars (Azerradj and Sigoise) elaborated as Spanish-style table olives were studied. The pH and the titratable acidity followed the same general pattern during the fermentation process for both cultivars but Sigoise reached higher titratable acidity and lower pH values. Both varieties exhibited a high total phenolic content of 5382.7 and 6754.3 mgGAE/100g and a total flavonoid of 424.6 and 568.5 mgRE/100g for the raw fruit of Azerradj and Sigoise, respectively, but decreased a 55.2 - 66.26% after 120 days of fermentation. The α -tocopherol decreased along the elaboration process while the β and γ -tocopherols remained constant. The losses of polyphenols and tocopherols were well correlated with the dropping of DPPH antiradical and the ferrous chelating activity during the processing. The fatty acid content was less affected by the processing than the other components without significant changes. The results of this work revealed that Sigoise cultivar, the most used, showed better nutritional values and suitability to the Spanish-style processing than Azerradj variety. However, table olives from both varieties can still be considered as a functional food with high amounts of bioactive compounds involved in health benefits.

Keywords: Table olives, Spanish-Style, Phenolic compounds, Tocopherols, Antioxidant activity.

1. INTRODUCTION

Olives are the fruits of the *Olea Europaea* L. tree, the most cultivated plant in the Mediterranean basin since the ancient time and is used mainly for the production of olive oil and table olives, which are one of the most important fermented vegetable foods in the Mediterranean countries [1].

Olive drupes have a bitter taste and need a treatment to hydrolyse the oleuropein, the main phenolic compound responsible for the bitterness. The methods used to obtain palatable olives are different and depend on the region, cultivar, the stage of maturity of the olives, fermentation conditions, autochthonous microbiota, chemical composition, strategies to debitter, and season [2]. The three main types of commercial table olives are Spanish-style green olives, California-style black olives and Greek-style natural black olives. In the Spanish-style elaboration, the olives are treated with lye to eliminate the bitter taste. Then, fruits are washed with water and put in brine to undergo a lactic fermentation for several months. The Californian style includes preservation in brine, lye treatment with air oxidation, washing, colour fixation by adding an iron salt and finally canning and sterilising. The production of naturally black olives in brine is milder but slower, and requires no chemicals, simply put in brine for several months (8-12 months) until debittering [3].

In recent years, consumers have changed their way of looking at food, not

only they are seeking for quality foods but also those that promote health and wellness. As a result of this change in habits, the Mediterranean diet has been trendy due to its low incidence of some coronary heart and breast, colon, and skin cancer [4].

Table olives, considered as an important source of nutrients, are an essential part of the Mediterranean diet which led to the increase of their consumption by 182% between 1990/91 and 2016/17 [5].

The benefits of table olives are attributed to some of their components; they are rich in monounsaturated fatty acids that reduce the risk of atherosclerosis, increase HDL-cholesterol, and decrease the complex LDL-cholesterol [6]. The minor compounds polyphenols, have been studied in depth during the last years due to their potent antioxidant activity displaying anticancer, anti-angiogenic and anti-inflammatory properties [7]. Furthermore, table olive is a source of many other natural compounds with physiological benefits like fibre, tocopherols, triterpenic acids and carotenoids. Table olives have also been suggested as suitable carriers for probiotics, especially for persons with lactose intolerant and low cholesterol diet needs [8].

Many studies have been carried out to investigate the influence of Spanish-style processing on different olives cultivar and fruit components. The chemical profile (sugars, organic acids, and volatile compounds) of industrial fermented green olives of Manzanilla, Hojiblanca, and Gordal cultivars was determined [9], and the Moroccan Picholine, Languedoc Picholine, Ascolana and Sevillana cultivars were studied [10]. The fat fraction of the Manzanilla and Hojiblanca was investigated [11, 12]. However, most of the papers only studied the effect of the elaboration process on the phenolic content [13-15]. To our knowledge, there is only one study carried out on Algerian cultivars [16] and is focused exclusively on the study of the phenolic compounds and the changes in antioxidant capacity at the raw stage and at the end of the fermentation.

This work aimed to characterise the chemical composition of two prominent Algerian cultivars, Sigoise and Azerradj, during the elaboration of table olives according to the Spanish-style method and evaluate the changes occurred on polyphenols, sugars, tocopherols, fatty acids and antioxidant activity during processing to provide information about how the processing influence on the nutritional value and quality of the table olives.

2. MATERIALS AND METHODS

2.1 OLIVE FRUITS PROCESSING AND SAMPLING

The fruits of two main Algerian olive cultivars harvested in their own traditional growing area were

used in this study; Sigoise region (north-west of Algeria) for Sigoise and Kabylie region (north east of Algeria) for Azzeradj.

The fruit of the Sigoise (fruit weight: 4.09 ± 0.55 g) and Azerradj cultivar (Fruit weight: 4.89 ± 0.78 g) were processed and fermented in a local plant in Seddouk (southeast of Bejaia province). Briefly, fresh and healthy olives were washed with water and placed into plastic screw-capped barrels of 200 L of capacity with a sodium hydroxide solution (2.5%, lye treatment) for 10 to 12 h until lye reached the 2/3 of the olive pulp. Afterward, fruits were washed three times with tap water every 4 h. Finally, fruits were covered with brine (10% NaCl solution) and left to ferment for several months. Samples of olive fruits and brines were collected for each cultivar at time 0 (raw olives) and after 10, 15, 20, 30, 40, 55, 70, 90 and 120 days of fermentation. The olives were destoned, freeze-dried, and stored at -20°C for further analysis.

2.2 DETERMINATION OF pH AND TITRATABLE ACIDITY (TA)

The pH values were measured using a Crison micro pH2000 pH meter. TA results were measured [17] and expressed as the percentage (% w/v) of lactic acid.

2.3 SUGARS ANALYSIS

Sugars and organic acids were extracted as described elsewhere [18]. One g of freeze-dried olive pulp was added to 20 mL of boiling water and shaken for 1 min, kept in an ultrasonic bath for 3 min, agitated for 1 min again, and centrifuged at 9000 g for 5 min. The mixture was filtered through a paper filter using vacuum, 20 mL of hot water was added and filtered again. The filtrate was then transferred to a 50 mL volumetric flask containing 2 mL of sorbitol as internal standard (7.5%, w/v) and made up to volume. The solution was kept at 5°C for 24 h to remove lipids and subsequently filtered through a $0.22 \mu\text{m}$ pore size nylon filter. Besides, for the sugar analysis, two millilitres of the clarified liquid were put into contact with 1 g of the acidic resin Amberlite IR-120 and 1 g of the basic resin Amberlite IRA-93. Samples were shaken occasionally for 30 min, and 1 mL of the solution was centrifuged at 9000 g for 3 min and filtered. The HPLC system used for the analysis of the sugars was the same as described [18].

2.4 TOCOPHEROLS ANALYSIS

The oil was cold extracted from previously crushed grains by shaking in iso-octane [19]. Analysis of tocopherols of oil solution was performed using an HPLC system (ThermoFinnigan, USA) equipped with a reversed-phase silica column Allsphere ODS2 ($5 \mu\text{m}$, $250 \text{ mm} \times 4.6 \text{ mm}$; Alltech, Belgium) and a photodiode array detector. A mobile phase

of acetonitrile/methanol (1:1) at a flow rate of 1.3 mL/min was used. The analysis was recorded at 292 nm. The different isomeric forms were identified comparing other vegetable oils typical for their tocopherol content distribution. The quantification was conducted using an external calibration solution of alpha-tocopherol in acetone (0.01 mg/mL) [20].

2.5. FATTY ACIDS ANALYSIS

Fatty acid composition of the oil extracted [19] (ISO 17059, 2009) was prepared as methyl-esters [21] and analysed with the 7890 Agilent gas chromatography instrument (Agilent, Germany) equipped with a FID detector. The experimental conditions used a capillary column HP88 Agilent 112-88177 (100 m × 0.25 mm, 0.20 µm). 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 quantified by comparing their retention times with those of standard compounds. Results were expressed in percentages of the total fatty acids.

2.6 DETERMINATION OF TOTAL POLYPHENOL AND FLAVONOID CONTENT

The olive extract was prepared according to the method described [16]. A quantity (1 g) of the lyophilised olive sample was mixed with 5 mL methanol and agitated for 20 min. The extract was then centrifuged at 3000 rpm/5 min and washed twice with hexane. The residue was extracted again twice in the same conditions, and then the extracts were combined and filtered.

Total phenolic compounds of olive extracts were determined according to the Folin-Ciocalteu procedure [22]. The results are expressed in mg of gallic acid equivalent (GAE) per 100g of dry weight (DW). The flavonoid content of the olive extract was estimated [23]. 0.5 mL of the olive extract was added to the same volume of aluminium chloride solution (2%). The absorbance was measured at 415 nm and the flavonoid content was expressed as mg of rutin equivalents (RE) /100 g DW.

2.7 DPPH FREE RADICAL SCAVENGING ACTIVITY

The antiradical activity of olive samples was determined [24]. 100 µl of the olive extract was mixed with 900 µl of a methanolic solution 60 µM DPPH (1, 1-diphenyl-2-picrylhydrazyl radical). The absorbance was measured at 517 nm and the antiradical activity was expressed as mg of Trolox equivalents (TE)/100 g of DW.

2.8 FERROUS-CHELATING ACTIVITY

The chelating power of ferrous ions (Fe²⁺) for the olive

extracts was measured following the protocol described [25]. 0.1 mL of olive extract was added to 0.05 mL of FeCl₂ (2 mM). The reaction was initiated by the addition of 0.1 mL of ferrozine (5 mM) and 2.75 mL of distilled water. The absorbance of the solution was measured at 562 nm. The scavenging activity was expressed as mmol of EDTA equivalents (EE)/100 g of DW.

2.9 STATISTICAL ANALYSIS

The results are given as the mean values of triplicates of the analysis and were subjected to the analysis of variance using the Statistica 5.0 software (StatSoft'97 edition) using the least significant difference (Newman-Keuls) test. Significance was defined at (p<0.05).

3. RESULTS AND DISCUSSION

The pH and the acidity of the final product are the most important parameters in the elaboration of the table olives. The drop off pH and the increase of the acidity determine the success of the fermentation [26]. The evolution of the pH (Figure 1) followed the same general trend for the two cultivars. pH dropped significantly during the first two weeks of brining and then decreased gradually until a final pH of 4.49 and 4.21 after 120 days of fermentation for Azerradj and Sigoise varieties, respectively. The decrease of the pH is mainly due to the transformation of the sugars, diffused from the fruits to the brine, into organic acids, especially lactic acid by lactic acid bacteria. Consequently, the titratable acidity (TA) was relatively stable (around 0.1%) during the first 20 days for both cultivars (Figure 1). After that, the TA increased higher for the Sigoise cultivar (0.59 %) than for Azerradj variety (0.23 %).

The primary sugar in raw olives is glucose, recording 68% of total sugar in Azerradj and 79% in Sigoise, followed by mannitol, fructose, and sucrose in less concentration (Table I). Lower sugar concentrations for the Hojiblanca cultivar but with the same distribution was noted [27], while Manzanilla-Aloreña cultivar showed a similar sugars profile [28]. The sugars monitored (Table I) showed a rapid decrease during the first 10 days of fermentation for both cultivars due to the loss of these compounds during the lye treatment and washing steps. Then, the sugars decreased slightly until the end of the fermentation. Glucose and sucrose showed the same patterns in both varieties, but not the fructose and mannitol which increased during the first month for Azerradj cultivar. The sugars of the fruits diffuse to the brine during the fermentation step and they are metabolised as substrates by fermentative microorganisms [28]. The glucose, fructose and sucrose are the most

readily substrate to be consumed by microorganisms [29]. This statement could explain the relatively high concentration of mannitol found in this work, as well as the commercial fermented olives analysed by Lopez-Lopez et al. [28]. Also, raw olives of cultivars with higher sugars concentration at the beginning of fermentation processing ensure a more acidic medium for better preservation [30]. Thus, the Sigoise cultivar showed a lower pH value and higher TA at the end of the fermentation.

Tocopherols play an essential role in the protection of the mono and polyunsaturated fatty acids from oxidation. As seen in Table II, the α -tocopherol is the most abundant tocopherol in the raw olives with initial values of 290.9 and 218.5 mg/kg for Sigoise and Azerradj cultivars, respectively, followed by γ -tocopherols and β -tocopherol in lower concentra-

tions (δ -tocopherol was not detected). These values were lower than the results found by Lainer et al. [31]. α -tocopherol decreased significantly to 36.3% for Azerradj and 38.6% for Sigoise, and no significant differences were found for the β -tocopherol and γ -tocopherols at the end of fermentation. This loss of α -tocopherol could be the consequence of the diffusion to the brine and its protective role on the unsaturated fatty acids against oxidation [27] and probably β -tocopherol and γ -tocopherols are less sensitive to the oxidation [32].

The fatty acid composition during table olive fermentation is shown in Table III. As expected for olives, the most abundant fatty acid is oleic acid [11] with 72.7% and 72.6% for raw fruits of Azerradj and Sigoise respectively, followed by palmitic acid (16.4-12.7%), linoleic acid (7.6-8.0%) and stearic acid (3.4-3.82%).

The changes in the fatty acid composition were limited to a slight decrease of the oleic acid by 2.1%, and the increase of the palmitic acid by 8.7% during the fermentation of the Azerradj cultivar. However, the composition of fatty acids for Sigoise variety was more affected by the processing. A decrease was observed for the margaric, heptadecenoic, stearic, oleic, linoleic, arachidic, and behenic and lignoceric acids. On the contrary, the stearic and palmitoleic acids increased. The decrease of fatty acids can be attributed to the alkali treatment or the diffusion to the brine [33], while the increase may be due to the method of calculation since the total of fatty acid must be 100%, which means that the decrease of a fatty acid implies the increase of at least one other

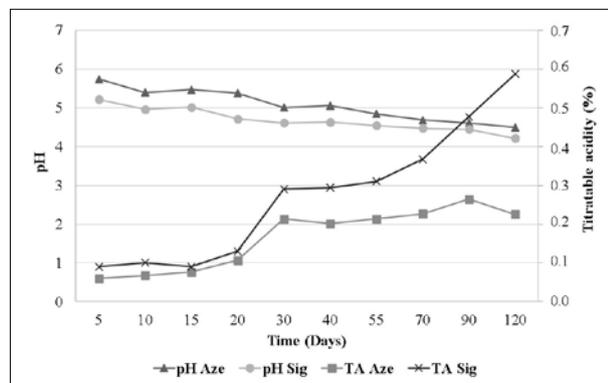


Figure 1 - Evolution of pH and titratable acidity (TA, % of lactic acid) during the table olives fermentation of Azerradj (Aze) and Sigoise (Sig) cultivars.

Table I - Sugars composition (g/kg) during table olives fermentation of Azerradj and Sigoise varieties.

Variety	Time (days)	Sucrose	Glucose	Fructose	Mannitol
Azerradj	0	2.5 ± 0.2 ^b	45.9 ± 3.7 ^b	7.7 ± 0.4 ^d	10.9 ± 0.2 ^{ab}
	10	0.9 ± 0.0 ^{cd}	29.4 ± 0.4 ^d	5.7 ± 0.1 ^{ef}	7.6 ± 0.6 ^{cd}
	15	0.6 ± 0.1 ^{def}	24.1 ± 0.3 ^f	5.7 ± 0.3 ^{ef}	5.8 ± 0.1 ^{def}
	20	0.5 ± 0.1 ^{ef}	22.9 ± 0.4 ^{fg}	5.9 ± 0.4 ^e	5.7 ± 0.2 ^{def}
	30	0.4 ± 0.1 ^{fg}	20.6 ± 0.4 ^g	5.5 ± 0.4 ^{ef}	5.3 ± 0.4 ^{def}
	40	0.0 ± 0.0 ^h	17.1 ± 0.2 ^h	5.4 ± 0.4 ^{ef}	5.2 ± 0.1 ^{def}
	55	0.0 ± 0.0 ^h	8.8 ± 0.8 ⁱ	4.1 ± 0.2 ^{fg}	4.4 ± 0.2 ^{def}
	70	0.0 ± 0.0 ^h	7.4 ± 1.3 ⁱ	3.4 ± 0.6 ^g	4.6 ± 0.5 ^{def}
	90	0.0 ± 0.0 ^{gh}	3.5 ± 0.0 ^j	2.9 ± 1.2 ^g	3.4 ± 0.4 ^f
120	0.0 ± 0.0 ^{gh}	3.2 ± 0.2 ^j	1.8 ± 0.10 ^h	3.6 ± 0.5 ^{ef}	
Sigoise	0	2.9 ± 0.3 ^a	57.7 ± 0.2 ^a	6.3 ± 0.9 ^e	5.8 ± 0.2 ^{def}
	10	1.0 ± 0.2 ^c	29.0 ± 0.2 ^d	5.2 ± 0.1 ^{ef}	6.9 ± 0.4 ^{cdef}
	15	1.0 ± 0.0 ^c	30.8 ± 1.0 ^{cd}	12.0 ± 0.3 ^b	8.6 ± 0.2 ^{bcd}
	20	1.0 ± 0.1 ^{cd}	30.8 ± 0.1 ^{cd}	12.7 ± 0.2 ^b	9.4 ± 1.4 ^{bc}
	30	0.9 ± 0.1 ^{cd}	26.5 ± 0.9 ^e	12.7 ± 0.3 ^b	12.8 ± 4.2 ^a
	70	0.8 ± 0.2 ^{cde}	32.3 ± 1.1 ^{cd}	15.9 ± 0.1 ^a	9.6 ± 0.1 ^{bc}
	55	0.6 ± 0.0 ^{cdef}	21.7 ± 0.0 ^g	9.7 ± 0.4 ^c	7.4 ± 0.0 ^{cde}
	70	0.0 ± 0.0 ^{gh}	17.5 ± 0.3 ^h	8.7 ± 0.3 ^{cd}	7.0 ± 0.1 ^{cdef}
	90	0.0 ± 0.0 ^h	10.2 ± 0.7 ⁱ	5.5 ± 1.4 ^{ef}	5.7 ± 0.6 ^{def}
120	0.0 ± 0.0 ^{gh}	9.4 ± 0.2 ⁱ	6.1 ± 0.1 ^e	6.6 ± 0.0 ^{cdef}	

Results are given as mean ± standard deviation. Averages in each column followed by different letters were significantly different at $p < 0.05$ according to a Newman and keuls test. The results are arranged in decreasing order; $a > b > c > d > e > f > g > h > i > j$

fatty acid [34]. Despite the significant differences found in the analysis of the fatty acid composition, no clear trends were observed due to the processing steps. The same conclusion during the processing of green Spanish-style table olives of Manzanilla and Hojiblanca cultivars was noted [11].

The total phenolic content (Table IV) showed a higher level of total polyphenols for the raw fruit than those found [16] for Azerradj (2406.8mg/100g DW) and Sigoise (1923.8 mg/100g DW). Values between 1900.0-2900.0mg/100g DW for Moroccan Picholine, Languedoc Picholine, Ascolana and Sevillana cultivars were also noted [10]. Nevertheless, Marsilio et al. [35] found the same range of phenolic content for Ascolana tenera green olives. The phenolic compounds decreased by 66.3% and 55.2% for Azerradj and Sigoise respectively after 120 days of fermentation and around half of this loss occurred during the first 10 days. The main changes in phenolic composition occur during the alkali treatment. Oleuropein, the main phenolic compound in olives, is hydrolyzed into hydroxytyrosol and elenolic acid glucoside and the bitterness of olives is removed. Hydroxytyrosol diffuses rapidly into the brine and remains at constant concentration while the oleuropein levels decrease probably due to the hydrolysis of oleuropein into oleoside-11-methyl ester and hydroxytyrosol [36]. The flavonoids were less affected directly by the alkali treatment and they decreased gradually throughout the fermentation from 424.5-568.4 mg RE/100g DW at the fresh stage to 131.6-279.3 mg RE/100gDW after 120 days of fermentation. The Sigoise cultivar showed a

higher total phenolic and flavonoid content than the Azerradj variety and therefore slightly higher resistance to alkali treatment. Mettouchi et al. [16] also found that the Sigoise is less affected by the treatment than Azerradj but with different loss percentages of 12.3% and 94.9% respectively.

The results of the DPPH free radical scavenging activity are presented in Table IV. The fresh olives displayed an antiradical activity of 1371.8 and 7063.0 mg TE/100g DW for Azerradj and Sigoise, respectively. The decrease of radical scavenging followed the same pattern as the phenolic content, with a high correlation ($r = 0.96$), but was less affected by the processing showing a loss of 38.1% and 42.8% for Azerradj and Sigoise respectively after 120 days of fermentation. This may be due to the presence of other compounds than polyphenols or to the polyphenols that are in the extract in an intermediate state of oxidation.

The ferrous chelating activity of the raw olives was 1.8 and 1.5 mmol EE/100g DW for Azerradj and Sigoise cultivars, respectively (Table IV). Also, this activity showed a similar trend than the phenolic content with a good correlation ($r = 0.82$). The ferrous chelating activity showed a quick drop during the two first weeks and continued decreasing slightly during the rest of the fermentation. After 120 days of fermentation, the ferrous chelating activity decreased by 60.0% and 57.8% for Azerradj and Sigoise, respectively (Table IV). The higher chelating activity of the Azerradj compared to the Sigoise suggest that all the activity cannot be ascribed exclusively to the phenolic content.

Table II - Evolution of tocopherols (mg/kg) during the table olive fermentation of Azerradj and Sigoise varieties.

Variety	Time (days)	Beta	Gamma	Alpha	Sum of tocopherols
Azerradj	0	0.3 ± 0.1 ^e	0.7 ± 0.1 ^b	218.1 ± 5.4 ^f	219.0 ± 5.4 ^f
	10	0.3 ± 0.1 ^{de}	0.7 ± 0.1 ^b	205.6 ± 1.7 ^g	206.5 ± 1.6 ^g
	15	0.4 ± 0.1 ^{cde}	0.7 ± 0.1 ^b	193.5 ± 4.0 ^h	194.5 ± 4.2 ^h
	20	0.3 ± 0.0 ^{de}	0.7 ± 0.1 ^b	186.6 ± 0.1 ^{hj}	187.6 ± 0.1 ^{hj}
	30	0.4 ± 0.1 ^{cde}	0.6 ± 0.4 ^b	178.8 ± 2.7 ^{ij}	179.8 ± 3.2 ⁱ
	40	0.4 ± 0.1 ^{cde}	0.7 ± 0.0 ^b	166.7 ± 1.5 ^k	167.7 ± 1.6 ^{ik}
	55	0.3 ± 0.1 ^{de}	0.8 ± 0.1 ^b	160.1 ± 7.7 ^k	161.1 ± 7.9 ^k
	70	0.3 ± 0.1 ^{de}	0.7 ± 0.0 ^b	158.8 ± 3.7 ^k	159.8 ± 3.7 ^k
	90	0.4 ± 0.1 ^{cde}	0.8 ± 0.1 ^b	153.1 ± 9.5 ^k	154.2 ± 9.5 ^k
120	0.6 ± 0.1 ^{abcde}	0.8 ± 0.1 ^b	138.6 ± 1.0 ^l	139.9 ± 1.0 ^l	
Sigoise	0	0.5 ± 0.0 ^{bcd}	0.8 ± 0.0 ^a	290.9 ± 2.2 ^a	292.2 ± 2.2 ^a
	10	0.6 ± 0.1 ^{abcde}	0.8 ± 0.1 ^a	288.1 ± 3.8 ^a	289.4 ± 3.7 ^a
	15	0.6 ± 0.0 ^{abcde}	0.9 ± 0.1 ^a	286.8 ± 3.9 ^a	288.2 ± 3.8 ^a
	20	0.7 ± 0.1 ^{abc}	0.8 ± 0.0 ^a	280.1 ± 5.4 ^{ab}	281.6 ± 5.3 ^{ab}
	30	0.6 ± 0.1 ^{abcde}	0.9 ± 0.2 ^a	272.8 ± 3.9 ^{bc}	274.2 ± 4.2 ^{bc}
	70	0.8 ± 0.2 ^{ab}	0.9 ± 0.2 ^a	265.8 ± 0.4 ^c	267.4 ± 0.4 ^c
	55	0.9 ± 0.1 ^a	0.9 ± 0.0 ^a	252.0 ± 1.4 ^d	253.8 ± 1.2 ^d
	70	0.7 ± 0.1 ^{abcd}	1.0 ± 0.1 ^a	244.2 ± 5.3 ^d	245.8 ± 5.3 ^d
	90	0.9 ± 0.2 ^{ab}	0.9 ± 0.1 ^a	232.2 ± 1.6 ^e	233.9 ± 1.3 ^e
120	0.8 ± 0.1 ^{ab}	0.7 ± 0.1 ^a	178.7 ± 12.3 ^j	180.2 ± 12.5 ^{ij}	

Results are given as mean ± standard deviation. Averages in each column followed by different letters were significantly different at $p < 0.05$ according to a Newman and keuls test. The results are arranged in decreasing order; $a > b > c > d > e > f > g > h > i > j > k > l$

Table III - Fatty acids composition (% of total fatty acids) during the table olive fermentations of Azerradij and Sigoise varieties.

Variety	Time (days)	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C24:0	Trans C18:1	
Azerradij	0	13.4 ± 0.4 ^e	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^b	0.3 ± 0.0 ^a	3.4 ± 0.1 ^e	72.7 ± 0.3 ^a	7.6 ± 0.0 ^{de}	0.6 ± 0.0 ^{bcd}	0.5 ± 0.0 ^{ab}	0.3 ± 0.0 ^{efg}	0.1 ± 0.0 ^a	0.0	
	10	13.4 ± 0.0 ^e	0.7 ± 0.0 ^{0g}	0.2 ± 0.0 ^a	0.3 ± 0.0 ^a	3.6 ± 0.0 ^{bcd}	72.5 ± 0.0 ^{abc}	7.6 ± 0.0 ^e	0.5 ± 0.0 ^{bcd}	0.6 ± 0.0 ^a	0.3 ± 0.0 ^{abcfg}	0.1 ± 0.0 ^a	0.0	
	15	13.4 ± 0.1 ^e	0.7 ± 0.0 ^{0g}	0.2 ± 0.0 ^a	0.3 ± 0.0 ^a	3.7 ± 0.0 ^{ab}	72.7 ± 0.1 ^a	7.3 ± 0.0 ^f	0.5 ± 0.0 ^{bcd}	0.6 ± 0.0 ^a	0.3 ± 0.0 ^{abcdef}	0.1 ± 0.0 ^a	0.0	
	20	13.6 ± 0.0 ^e	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^{ab}	0.3 ± 0.0 ^a	3.7 ± 0.0 ^{bc}	72.2 ± 0.0 ^c	7.7 ± 0.0 ^{de}	0.5 ± 0.0 ^{bcd}	0.6 ± 0.0 ^a	0.3 ± 0.0 ^{abcdef}	0.1 ± 0.0 ^a	0.0	
	30	13.5 ± 0.0 ^e	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^{ab}	0.3 ± 0.0 ^a	3.7 ± 0.0 ^{bc}	72.3 ± 0.0 ^{bc}	7.7 ± 0.0 ^{de}	0.5 ± 0.0 ^{bcd}	0.6 ± 0.0 ^a	0.3 ± 0.0 ^{abcfg}	0.1 ± 0.0 ^a	0.0	
	40	13.4 ± 0.1 ^e	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^{ab}	0.3 ± 0.0 ^a	3.6 ± 0.0 ^{cde}	72.3 ± 0.1 ^{bc}	7.8 ± 0.0 ^{bcd}	0.5 ± 0.0 ^{bcd}	0.6 ± 0.0 ^a	0.3 ± 0.0 ^{abcdef}	0.1 ± 0.0 ^a	0.0	
	55	13.5 ± 0.1 ^e	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^b	0.3 ± 0.0 ^a	3.4 ± 0.0 ^{de}	72.4 ± 0.1 ^{abc}	7.7 ± 0.0 ^{de}	0.5 ± 0.0 ^{bcd}	0.6 ± 0.0 ^{ab}	0.3 ± 0.0 ^{abcdef}	0.1 ± 0.0 ^a	0.0	
	70	13.7 ± 0.2 ^e	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^b	0.3 ± 0.0 ^a	3.5 ± 0.0 ^{cde}	72.0 ± 0.1 ^d	8.0 ± 0.0 ^{abc}	0.6 ± 0.0 ^d	0.5 ± 0.0 ^{ab}	0.3 ± 0.0 ^{aeffg}	0.1 ± 0.0 ^a	0.0	
	90	14.0 ± 0.4 ^d	0.8 ± 0.0 ^f	0.2 ± 0.0 ^b	0.3 ± 0.0 ^a	3.5 ± 0.1 ^{cde}	71.9 ± 0.3 ^{de}	7.8 ± 0.0 ^{de}	0.5 ± 0.0 ^{bcd}	0.5 ± 0.0 ^{ab}	0.3 ± 0.0 ^{fg}	0.1 ± 0.0 ^a	0.0	
	120	14.6 ± 0.1 ^c	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^b	0.3 ± 0.0 ^a	3.5 ± 0.0 ^{de}	71.2 ± 0.1 ^f	7.9 ± 0.0 ^{abc}	0.5 ± 0.0 ^{bcd}	0.6 ± 0.0 ^a	0.3 ± 0.0 ^{abcde}	0.1 ± 0.0 ^a	0.0	
	Sigoise	0	12.7 ± 0.0 ^f	0.8 ± 0.0 ^{fg}	0.2 ± 0.0 ^{ab}	0.3 ± 0.0 ^a	3.8 ± 0.0 ^e	72.6 ± 0.1 ^{abc}	8.0 ± 0.1 ^{abc}	0.6 ± 0.0 ^{ab}	0.6 ± 0.0 ^{ab}	0.3 ± 0.0 ^{abcdef}	0.1 ± 0.0 ^a	0.0
		10	13.2 ± 0.0 ^e	0.9 ± 0.0 ^{0e}	0.2 ± 0.0 ^c	0.3 ± 0.0 ^a	3.2 ± 0.0 ^f	72.7 ± 0.1 ^a	8.0 ± 0.0 ^{abc}	0.6 ± 0.0 ^{ac}	0.5 ± 0.0 ^b	0.3 ± 0.0 ^{efg}	0.1 ± 0.0 ^a	0.0
15		14.2 ± 0.1 ^d	1.7 ± 0.0 ^{0d}	0.1 ± 0.0 ^e	0.2 ± 0.0 ^c	2.6 ± 0.0 ^{0g}	71.6 ± 0.0 ^e	8.0 ± 0.1 ^{ab}	0.7 ± 0.1 ^a	0.5 ± 0.0 ^c	0.3 ± 0.0 ^{bcd}	0.1 ± 0.0 ^a	0.0	
20		15.2 ± 0.1 ^b	1.8 ± 0.1 ^c	0.1 ± 0.0 ^{fg}	0.2 ± 0.0 ^d	2.5 ± 0.2 ^{gh}	70.6 ± 0.2 ^g	8.1 ± 0.2 ^a	0.7 ± 0.1 ^a	0.4 ± 0.0 ^{cd}	0.3 ± 0.0 ^{abcde}	0.1 ± 0.0 ^a	0.0	
30		15.3 ± 0.0 ^{ab}	2.1 ± 0.0 ^{0b}	0.1 ± 0.0 ^f	0.1 ± 0.0 ^f	2.4 ± 0.1 ^h	70.4 ± 0.1 ^g	8.0 ± 0.1 ^{ab}	0.7 ± 0.0 ^a	0.4 ± 0.0 ^{de}	0.3 ± 0.0 ^{abcde}	0.1 ± 0.0 ^a	0.0	
40		15.5 ± 0.0 ^{ab}	1.9 ± 0.0 ^{0b}	0.1 ± 0.0 ^g	0.1 ± 0.0 ^e	2.4 ± 0.0 ^h	70.4 ± 0.1 ^g	8.0 ± 0.0 ^{abc}	0.7 ± 0.0 ^a	0.5 ± 0.0 ^{cd}	0.3 ± 0.0 ^{abcd}	0.1 ± 0.0 ^a	0.0	
55		15.7 ± 0.0 ^a	1.9 ± 0.0 ^{0b}	0.1 ± 0.0 ^h	0.1 ± 0.0 ^e	2.5 ± 0.1 ^{gh}	70.3 ± 0.1 ^g	7.9 ± 0.0 ^{abc}	0.7 ± 0.0 ^a	0.4 ± 0.0 ^e	0.3 ± 0.0 ^{0g}	0.0 ± 0.0 ^b	0.0	
70		15.6 ± 0.1 ^{ab}	1.7 ± 0.0 ^{cd}	0.1 ± 0.0 ^f	0.1 ± 0.0 ^e	2.5 ± 0.0 ^{gh}	70.2 ± 0.1 ^g	8.0 ± 0.0 ^{abc}	0.7 ± 0.0 ^a	0.5 ± 0.0 ^{cd}	0.3 ± 0.0 ^d	0.1 ± 0.0 ^a	0.0	
90		15.6 ± 0.1 ^a	1.7 ± 0.0 ^{0d}	0.1 ± 0.0 ^d	0.2 ± 0.0 ^b	2.6 ± 0.0 ^{4g}	70.3 ± 0.1 ^g	7.9 ± 0.0 ^{abc}	0.6 ± 0.0 ^a	0.4 ± 0.0 ^{cd}	0.3 ± 0.0 ^{abcdfg}	0.1 ± 0.0 ^a	0.0	
120		15.8 ± 0.1 ^a	1.9 ± 0.0 ^{0b}	0.1 ± 0.0 ^{ef}	0.1 ± 0.0 ^{de}	2.4 ± 0.0 ^h	70.2 ± 0.1 ^g	7.8 ± 0.0 ^{bcd}	0.7 ± 0.0 ^a	0.5 ± 0.0 ^{cd}	0.3 ± 0.0 ^{bcd}	0.1 ± 0.0 ^a	0.0	

Results are given as mean ± standard deviation. Averages in each column followed by different letters were significantly different at $p < 0.05$ according to a Newman and keuls test. The results are arranged in decreasing order, $a > b > c > d > e > f > g > h$

4. CONCLUSION

The results obtained in this work showed the significant loss of some of their components such as polyphenols, tocopherols and sugars during the Spanish-style processing of green olives of Azerradj and Sigoise varieties, while other compounds like fatty acids are less affected. The first steps in the elaboration process (lye treatment and washing) caused the most critical depletion of the nutritional components and was the Sigoise cultivar the one that showed a better nutritional value and good suitability to the Spanish-style processing than Azerradj table olives. However, table olives from both varieties can still be considered as a functional food with high

amounts of bioactive compounds. The balanced fatty acids composition and the high content of polyphenolic compounds can be involved in health benefits and exert an excellent antioxidant activity.

Acknowledgments

The authors wish to thank the Algerian Ministry of Higher Education and Scientific Research for sponsoring this work. The authors are grateful to the staff of the KHODJA & CO Company, Seddouk (Bejaia, Algeria), for providing the samples.

Conflicts of interest: there are no conflicts of interest to declare.

Table IV - Composition of total polyphenols (mg GAE/ 100 g DW), total flavonoids (mg RE/ 100 g DW), antiradical activity (mg TE/ 100 f DW) and ferrous-chelating activity (mmol EE/ 100 g DW) during table olive fermentation of Azerradj and Sigoise varieties.

Variety	Time (days)	Total polyphenols	Total flavonoids	Antiradical activity	Ferrous-chelating activity
Azerradj	0	5382.6±6.99 ^b	424.6 ± 10.8 ^b	1371.9 ± 13.3 ^e	1.8 ± 0.0 ^a
	10	2678.7 ± 83.4 ^e	357.7 ± 3.1 ^c	1131.3 ± 54.9 ^e	1.7 ± 0.0 ^b
	15	2133.8 ± 94.0 ^f	266.1 ± 11.7 ^{efg}	1009.5 ± 43.6 ^e	1.8 ± 0.0 ^{ef}
	20	1861.0 ± 3.5 ^g	257.3 ± 9.5 ^{efg}	992.4 ± 54.6 ^e	1.0 ± 0.0 ^{fg}
	30	1920.5 ± 20.7 ^g	245.8 ± 1.5 ^{fg}	974.3 ± 11.3 ^e	1.0 ± 0.0 ^{fg}
	40	1874.1 ± 42.1 ^g	223.8 ± 5.6 ^g	926.4 ± 11.5 ^e	1.1 ± 0.0 ^{ef}
	55	1878.4 ± 51.8 ^g	180.4 ± 5.5 ^h	831.7 ± 1.9 ^e	0.8 ± 0.0 ^{hi}
	70	1839.8 ± 125.9 ^g	145.5 ± 13.0 ⁱ	808.3 ± 83.8 ^e	0.8 ± 0.0 ^{hi}
	90	1847.6 ± 17.2 ^g	141.9 ± 1.2 ^{hi}	856.6 ± 15.0 ^e	0.8 ± 0.0 ^{hij}
	120	1816.0 ± 127.2 ^g	131.6 ± 10.6 ⁱ	847.6 ± 7.5 ^e	0.7 ± 0.0 ^{hijk}
Sigoise	0	6754.3 ± 49.1 ^a	568.5 ± 3.9 ^a	7063.0 ± 15.4 ^a	1.5 ± 0.0 ^c
	10	3390.3 ± 24.4 ^c	541.0 ± 22.3 ^a	4358.1 ± 107.0 ^{bcd}	1.1 ± 0.0 ^{de}
	15	3432.5 ± 83.6 ^c	422.5 ± 3.8 ^b	4704.6 ± 107.1 ^{bc}	1.2 ± 0.1 ^d
	20	3458.8 ± 52.2 ^c	409.3 ± 7.7 ^b	4502.6 ± 15.3 ^{bcd}	1.0 ± 0.0 ^g
	30	3458.5 ± 84.0 ^c	421.2 ± 5.4 ^b	4965.8 ± 568.6 ^b	1.0 ± 0.0 ^g
	70	3254.0 ± 253.9 ^{cd}	355.2 ± 18.4 ^c	4710.2 ± 183.3 ^{bcd}	0.8 ± 0.0 ^h
	55	3395.4 ± 48.9 ^{cd}	323.1 ± 17.7 ^{cd}	4617.2 ± 0.0 ^{bcd}	0.8 ± 0.0 ^h
	70	3066.5 ± 48.7 ^d	301.6 ± 53.0 ^{de}	4118.5 ± 76.4 ^{cd}	0.7 ± 0.0 ^{jk}
	90	3274.2 ± 24.3 ^{cd}	269.7 ± 9.2 ^{efg}	4050.4 ± 182.8 ^d	0.7 ± 0.0 ^{jk}
	120	3025.9 ± 143.0 ^d	279.4 ± 8.5 ^{ef}	4040.4 ± 628.1 ^e	0.7 ± 0.0 ^k

Results are given as mean ± standard deviation. Averages in each column followed by different letters were significantly different at $p < 0.05$ according to a Newman and keuls test. The results are arranged in decreasing order; a > b > c > d > e > f > g > h > i > j > k
GAE: gallic acid equivalent; RE rutin equivalents; TE: Trolox equivalents; EE: EDTA equivalents; DW: Dry weight

REFERENCES

- [1] A. Pasqualone, R. Nasti, C. Montemurro, T.Gomes, Effect of natural style processing on the oxidative and hydrolytic degradation of the lipid fraction of table olives. *Food control* 37, 99-103 (2014).
- [2] M. Alves, C. Quintas, Traditional Green Table Olives from the South of Portugal. In: Kristbergsson K., Oliveira J. (Eds.) *Traditional foods. Integrating food science and engineering knowledge into the food chain* 10 (2016). Springer, Boston, MA.
- [3] A.H. Sánchez Gómez, P. García, L. Rejano Navarro, Elaboration of table olives. *Grasas y Aceites* 57, 86-94 (2006).
- [4] F. Visioli, A. Poli, C. Galli, Antioxidant and other biological activities, of phenols from olives and olive oil. *Medicinal Research Reviews* 22(1), 65-75, (2002).
- [5] International Olive Oil Council (IOC) Updates Series of World Statistics on Production, Imports, Exports and Consumption. [(accessed on 30 September 2019)]. 2018.

Available online:

<http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures>

- [6] E. Tripoli, M. Giammanco, G. Tabacchi, D. Di Majo, S. Giammanco, M.J. La Guardia, The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutrition Research Review* 18(1), 98-112 (2005).
- [7] M. Gorzynik-Debicka, P. Przychodzen, F. Cappello, A. Kuban-Jankowskan, A. Marino Gammazza, N. Knap, M. Wozniak, Potential health benefits of olive oil and plant polyphenols. *International Journal of Molecular Sciences* 19(3), 686 (2018).
- [8] C. Randazzo, A. Todaro, A. Pino, I. Pitino, O. Corona, A. Mazzaglia, C. Caggia, Giarrappa and Grossa di Spagna naturally fermented table olives: Effect of starter and probiotic cultures on chemical, microbiological and sensory traits. *Food Research International* 62, 1154-1164 (2014).
- [9] A. Montano, A. Sánchez, F. Casado, A. De Castro, L. Rejano, Chemical profile of industrially fermented green olives of different varieties. *Food Chemistry* 82(2), 297-302 (2003).
- [10] H. Kiai, A. Hafidi, Chemical composition changes in four green olive cultivars during spontaneous fermentation. *LWT-Food Science and Technology* 57(2), 663-670 (2014).
- [11] A. Lopez-Lopez, A. Cortés-Delgado, A. Garrido-Fernandez, Effect of green Spanish style processing (Manzanilla and Hojiblanca) on the quality parameters and fatty acids and triacylglycerol compositions of olive fat. *Food Chemistry* 188, 37-45, (2015).
- [12] A. López-López, A. Cortés-Delgado, A. Garrido-Fernández, Assessment of the Minor-Component Transformations in Fat during the Green Spanish-Style Table Olive Processing. *Journal of agricultural and food chemistry* 66(17), 4481-4489, (2018).
- [13] M. Brenes, L. Rejano, P. Garcia, A.H. Sanchez, A. Garrido, Biochemical changes in phenolic compounds during Spanish-style green olive processing. *Journal of agricultural and food chemistry* 43(10), 2702-2706 (1995).
- [14] N.B. Ben Othman, D. Roblain, N. Chammen, P. Thonart, M. Hamdi, Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. *Food Chemistry* 116(3), 662-669(2009).
- [15] R. Ambra, F. Natella, C. Bello, S. Lucchetti, V.Forte, G. Pastore, Phenolics fate in table olives (*Olea europaea* L. cv. Nocellara del Belice) debittered using the Spanish and Castelvetro methods. *Food Research International* 100, 369-376, (2017).
- [16] S. Mettouchi, R. Sacchi, Z.E.D. Ould Moussa, A. Paduano, M. Savarese, A. Tamendjari, Effect of Spanish style processing on the phenolic compounds and antioxidant activity of Algerian green table olives. *Grasas y Aceites* 67, 114-125, (2016).
- [17] A. Pino, M. De Angelis, A. Todaro, K. VanHoorde, C.L. Randazzo, & C. Caggia, Fermentation of Nocellara Etna table olives by functional starter cultures at different low salt concentrations. *Frontiers in microbiology* 9, 1125 (2018).
- [18] E. Medina, M. Brenes, C. Romero, A., García, A. de Castro, Main antimicrobial compounds in table olives. *Journal of Agricultural and Food Chemistry* 55(24), 9817-9823, (2007).
- [19] ISO 17059 Oilseeds - extraction of oil and preparation of methyl esters of triglyceride fatty acids for analysis by gas chromatography (rapid method), 1-5, (2009).
- [20] P. Rovellini, M. Azzolini, N. Cortesi, Tocoferoli e Tocotrienoli in oli e grassi vegetali. *Riv. Ital. Sostanze Grasse* 74, 1-5, (1997).
- [21] Commission Implementing Regulation (EU) No 1348/2013 of 16 December 2013, amending Regulation (EEC) No 2568/91 (2013) on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of European Union L* 338, 31-67.
- [22] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture* 16(3), 144-158, (1965).
- [23] A. Meda, C.E. Lamien, M. Romito, J. Millogo, O.G. Nacoulma, Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry* 91(3), 571-577, (2005).
- [24] L. Lesage-Meessen, D. Navarro, S. Maunier, J. Sigoillot, J. Lorquin, M. Delattre, M. Labat, Simple phenolic content in olive oil residues as a function of extraction systems. *Food Chemistry* 75(4), 501-507, (2001).
- [25] S. Bourgou, R. Ksouri, A. Bellila, I. Skandrani, H. Falleh, B. Marzouk, Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. *Comptes Rendus Biologies* 331(1), 48-55, (2008).
- [26] F. Sakouhi, S. Harrabi, C. Absalon, K. Sbei, S. Boukhchina, H. Kallel, α -Tocopherol and fatty acids contents of some Tunisian table olives (*Olea europea* L.): Changes in their composi-

- tion during ripening and processing. *Food Chemistry* 108, 833-839, (2008).
- [27] A. Garrido, P. García, A. Montaña, M. Brenes, M. Durán, Biochemical changes during the preservation stage of ripe olive processing. *Die Nahrung* 37(6), 583-591, (1993)
- [28] A. López-López, A. Jiménez-Araujo, P. García-García, A. Garrido-Fernández, Multivariate analysis for the evaluation of fibre, sugars, and organic acids in commercial presentations of table olives. *Journal of agricultural and food chemistry* 55(26), 10803-10811, (2007).
- [29] A. Garrido-Fernandez, M.J. Fernandez Diez, M.R. Adams, *Table Olives. Production and processing*. London, UK: Chapman & Hall (1997).
- [30] M. Maldonado, C. Zuritz, M. Assof, Diffusion of glucose and sodium chloride in green olives during curing as affected by lye treatment. *Journal of food engineering* 84(2), 224-230, (2008).
- [31] F. Lainer, N. Iaccarino, J. Amato, B. Pagano, A. Pagano, G. Tenore, G. Bellan, Characterization of monovarietal extra virgin olive oils from the province of Béjaïa (Algeria). *Food Research International* 89, 1123-1133, (2016).
- [32] F. Shahidi, A.C. de Camargo, Tocopherols and tocotrienols in common and emerging dietary sources: occurrence, applications, and health benefits. *International journal of molecular sciences* 17(10), 1745 (2016).
- [33] Y. Sahan, A. Cansev, H. Gulen, Effect of processing techniques on antioxidative enzyme activities, antioxidant capacity, phenolic compounds, and fatty acids of table olives. *Food Science and Biotechnology* 22(3), 613-620, (2013).
- [34] Garrido-Fernández, A. Cortés-Delgado, A. López-López, Tentative application of compositional data analysis to the fatty acid profiles of green Spanish-style Gordal table olives. *Food Chemistry* 241, 14-22, (2018).
- [35] V. Marsilio, C., Campestre, B. Lanza, Phenolic compounds change during california-style ripe olive processing. *Food Chemistry* 74(1), 55-60, (2001).
- [36] A. Montano, A.H. Sánchez, A. López-López, A. de Castro, L. Rejano, Chemical composition of fermented green olives: acidity, salt, moisture, fat, protein, ash, fiber, sugar, and polyphenol. *Olives and olive oil in health and disease prevention*. Elsevier, 291-297, (2010).