Effect of natural style processing on the lipid fraction of Sigoise turning colour table olives

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This work studies the effect of natural-style processing on the degradation of the lipid fraction of Algerian *Sigoise* turning colour table olives. Turning olives were directly fermented in brine. Samplings were carried out at harvest and after 60, 120 and 150 days of fermentation. The parameters of quality (acidity, peroxide value, K_{232}, and K_{270}), as well as hexanal and nonanal contents showed a moderate increase. Processing did not cause any systematic effect on fatty acids, triglycerides and total diglycerides, whereas, 1, 2-diglycerides decrease by isomerisation to 1, 3 diglycerides. Among the antioxidants of the oil, tocopherols were less affected than the phenolic compounds that undergo reduction and oxidation. As a result, the antioxidant activity against DPPH of the whole lipid fraction was less affected than its methanolic extract. Globally, this study showed a limited degradation of lipid fraction with processing, *Sigoise* Table olives (turning colour) elaborated by natural style can be considered as good functional food that could maintain a maximum of benefit components.

Keywords: Table olives, Natural style, Lipid fraction, Antioxidant activity.

1. INTRODUCTION

Table olives and olive oil are the basic component of the Mediterranean diet with important biological properties. The content of unsaturated fatty acids rich in oleic acid and phenolic compounds is responsible for the positive effects on health reduction of cardiovascular and cancerous diseases [1]. Algeria is one of the major olive-producing countries; olive tree ranked first amongst the fruit trees. Algerian production of table olives is 293.000 tons, which represent 10% of world production [2].

The olive fruit is mainly composed of water and lipids. The oil content in the olive fruit ranges from 14 to 30%, depending on the cultivar and ripening stage [3]. Phenolic compounds represent up to 2-3% of olive flesh. Oleuropein is the major phenolic bioactive compound responsible for the strong bitter and pungent taste that can be removed by natural methods, alkali treatment, drying, or salt curing. There are many types of table olives that differ by their method of debittering. The three main types of commercial table olives are: Spanish-style green olives, California-style black ripe olives and Greek- style natural black olives. The latter also referred to as natural-style or "natural method" because it does not use chemicals [4].The "natural olives", according to the "Trade Standard Applying to Table Olives" (IOC, 2004) are "green olives, olives turning colour or black olives placed directly in brine in which they undergo complete or partial fermentation, preserved or not by the addition of acidifying agents" [5].

During processing, physical, chemical and biochemical changes occur in olives. Many studies have been carried out regarding the influence of different

processing methods of table olives on the levels of total and single phenolics [6-9], sugar composition [10], fatty acids and triglycerides [11, 12], tocopherols [13], and volatile compounds [14-16]. Studies indicate that turning colour olives produced using natural methods have higher levels of phenolic compounds as compared to those produced using Spanish processing methods [6, 17]. However, a few works have been devoted to the effects of different style processing on the lipid fraction [11, 12, 18], particularly table olives prepared according to the natural style [19]. The natural style influences significantly the increase of the degradation of lipid fraction of black ripe table olives of Italian cultivars, but it limits the extent of the primary and secondary oxidation [19]. This style processing is characterised by its long duration that can influence the quality of the lipid fraction, which is the major component of table olives after water. The purpose of this work was to evaluate the effect of natural-style processing on degradation of the lipid fraction (quality parameters, di and triglycerides, fatty acids, tocopherols, volatile compounds, polyphenols and antioxidant capacity) of olives turning colour of *Sigoise* variety that is the main variety used for the production of table olives in Algeria.

2. MATERIALS AND METHODS

PROCESSING AND SAMPLING

The Algerian varieties of *Sigoise* olives (average weight 3.5 g) were harvested at the turning colour stage during the season (2015/2016). These samples were collected and processed according to the natural style; olives (two sub-lots of about 15 kg per trial) were processed twice separately in plastic tanks with a capacity of 30 L with 11% (w/v) NaCl of brine solution and left at room temperature to follow spontaneous fermentation for 5 months. The olives were maintained submerged in the brine. During the process period, from the beginning until having a pH of 4.3 of the brine solution, four Samplings were collected: raw *Sigoise* turning olives are the fresh fruits (at harvest), samples made after 60 days of fermentation, after 120 days and after 150 days.

ANALYSES ON OLIVES

Weight, moisture [20], and oil content of olives [21] was determined:

Oil extraction

Cold extraction of the oil was carried out by an oleodoseur (Levi-Dilon-Lerogsame) that consists in a centrifuge divider (3000 rpm). 3 Kg of turning Sigoise table olives were ground by a crusher, kneaded for 45min and then centrifuged to extract the oil. The oil was transferred into dark glass bottles and stored at 4°C until the analysis.

CHEMICAL CHARACTERISTICS OF THE OIL FRACTION

Oil quality index

Acidity, Peroxide Value (PV) and UV spectrophotometric indices (measured at 232 and 270 nm) were determined according to the analytical methods described by the European Union regulation [21].

Fatty acids compounds

The fatty acid composition was determined as methyl ester derivatives by gas chromatography (GC) instrument (7890 Agilent gas chromatography) equipped with an FID detector and a split/split less injector. Fatty acids methyl esters were prepared by vigorous shaking of the oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium hydroxide according to methods described in EEC Regulation (Commission regulation (ECC) 1991) and Commission Implementing Regulation (EU) 2015) [22]. The experimental conditions used were: 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 the 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.

Tri and Diglycerides of oil of table olives

Diglycerides and triglycerides composition were performed according to the IOC method [23]. One hundred milligrams of olive oil were weighed in a glass bottom conical tube and 1 ml of internal (dinonadecano in 0.1% w/v in heptane) was added. The mixture was stirred until a complete solution was obtained. Thirty microliters of solution were placed in a new glass tube (with a stopper) and dried by a gentle nitrogen stream. Two hundred µl of silylation reagent were added allowing the mixture to stand until silylation is complete. The solution was dried by a soft nitrogen flow. 2 ml of n-heptane were added and after shaking, 1 µl of solution, were injected in GC system. The injector temperature was left be at least 10°C below the vaporization temperature (99°C) of the employed solvent (n-Heptane). The column (Mega SE52 5 m + 2 m precolumn id. 0.32 mm 0.10 µm) was subjected to a temperature gradient: 1 min at 80°C, from 80°C to 220°C at 20°C/min, from 220 to 340°C at 5°C/min, 10 min at 340°C. The detector

FID temperature was 350°C. Helium was used as the carrier gas. The triglycerides and diglycerides peaks identification was carried out from the retention times by comparing them with mixtures of known composition. Results are expressed as percentage.

Carbonylic volatile compounds

Two volatiles compounds (Hexanal and Nonanal) were derivatised with 2.4 dinitrophenylhydrazine and determined by HPLC system. Four hundred milligrams of olive oil weighed in Schott test tube of 5 ml, 100 µl of internal standard solution in hexane (0.5 mg dodecanal/ml of hexane) was added. The mixture was mixed on Vortex for some second. 1 ml of 2, 4 dinitrophenylhydrazine (0.1% in acetonitrile 0.01N ($HClO_4$)) was added. The reaction was immediate and the carbonylic compounds derived were stable. The mixture was mixed on Vortex for 1 minutes and left to react in ultrasonic bath for 15 minutes at least. Then the solution was centrifuged at 5000 rpm for 15 minutes. The acetonitrile phase (5 µl) was injected in the HPLC system constituted by a quaternary gradient pump P4000 (ThermoFinnigan) and spectrophotometric detector UV6000LP (ThermoFinnigan). Chromatographic column was a reverse phase C18Spherisorb ODS2 3µm, l=25cm, i.d. = 4.0mm (Chrom-Germany). The mobile phase of the system was constituted by 45%-A (water), 20%-B (acetonitrile), 35%-C (methanol) with a linear gradient for 60 minutes to 0%-A, 50%-B, 50%-C. The low rate was 1 ml/min. The hexanal and nonanalaldehydes were quantified by measuring the peak area recorded at 360 nm and expressed as dodecanal in mg/kg of oil [24].

Tocopherols

Tocopherols composition was evaluated using an HPLC linked to a PDA (Photodiode Array Detector). A reversed phase silica column (Allsphere ODS2 (Alltech) 5 µm, 250 mm × 4.6 mm) was eluted with acetonitrile/methanol (1/1) at a flow rate of 1.3 ml/min. 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 utilising an external calibration solution of alpha-tocopherol in acetone (0.01 mg/ml) [25].

ANALYSIS OF PHENOLIC COMPOUNDS OF THE LIPID FRACTION

Total phenolic compounds

The extractions of polyphenols were performed according to the method described by Ollivier et al. [26] with slight modifications. Five grams of filtered oil were dissolved in 5ml of MeOH/H_2O (80/20), the mixture was vortexed and centrifuged at 3800 rpm during 15min. The polar fraction was transferred in a flask and the extraction was repeated three times until the final volume of 15ml.

The total phenolic content was determined using the Folin–Ciocalteu reagent as reported [27] with slight modification. In a 20 ml volumetric flask, a volume of 0,5ml of Folin-Ciocalteu reagent was added to 1mL of the phenolic extract and 5ml of distilled water. After 3 min, 4 ml of a sodium carbonate solution (Na_2CO_3) (10%) was added, and the total volume was adjusted with distilled water to 20ml. After 90 min of incubation in the dark, the solution was centrifuged, and the absorbance was read at 765 nm. The total phenol content was expressed in mg equivalent of gallic acid per kilogram of oil (mg GAE/kg) from a calibration curve (y= 5.0766x; R^2= 0.99).

Phenolic profile by HPLC

A solution of internal standard (1ml of 0.015 mg/ml of syringic acid in water/methanol (20/80 v/v) was added to the sample of extra virgin olive oil (2g). After shaking the mixture by vortex during 30s, 5 ml of extraction solution of water methanol (20:80 v/v) were added. The obtained mixture was mixed on a Vortex for 1 minute, extracted for 15 min in an ultrasonic bath and then centrifuged at 5000 rpm for 25 minutes (COI/T.20 Doc. N.29) [28]. The upper phase was filtered with a 0.45 µm PVDF syringe filter, 20µL of the filtered solution were analysed by HPLC with a UV detector selected at 280 nm and 240 nm. The HPLC separation was conducted by a system consisting of a C18 Spherisorb ODS-2 reversed column (5mm, 250 mm, id. 4.6 mm). Elution was performed at a flow rate of 1ml/min following a gradient, composed of a mixture of water and orthophosphoric acid (99.8: 0.2 v/V) (solvent A), methanol (solvent B) and acetonitrile (solvent C): from 96% (A) – 2% (B) – 2% (C) to 0% (A) – 50% (B) – 50% (C) in 60 minutes. The last gradient composition was kept for 10 minutes. Successively, it returned to the initial conditions of 95% (A) – 2% (B) – 2% (C) in 2 minutes and then maintained for 10 minutes [29]. The main phenolic compounds were identified in comparison with relative retention times and UV spectra of pure standards

ANTIOXIDANT ACTIVITY

Radical scavenging activity (RSA) of oil against DPPH radical

During the oxidation test, the presence of RSA and hydrogen donors in olive oil was tested by reduction of DPPH in toluene. Procedure reported [30] was adapted. The fresh DPPH toluene solution was prepared at a concentration of (10^{-4} M). 3.9ml of fresh DPPH solution was mixed with 1ml of diluted

oil in toluene. The mixture was vortexed for 20s at room temperature. Against a blank of toluene without DPPH•, the absorption was measured at 515 nm after 60mn of incubation.

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 [31]. Two millilitres of the methanolic extract was added to 2ml of DPPH solution prepared at 10^{-4} mM in methanol. The absorbance was measured at 515 nm after 30 mn of incubation in the dark. The result of radical scavenging activity was expressed as mg gallic acid equivalent per kilogram of oil (GAE/kg).

Statistical analysis

All the results are reported as the mean values (n = 3) and were subjected to analysis of variance using the Statistica 5.0 package (StatSoft'97 edition) with the least significant difference (Newman–Keuls) test. Significance was defined at ($p < 0.05$).

3. RESULTS AND DISCUSSION

In addition to several artisanal methods, three main processing methods, namely Spanish, Californian and Greek, were used to debitter olives. The first two processes that are the most important commercially use NaOH to hydrolyse the bitter compounds, whereas in the third method also known as "Natural olives," the olives are put in brine and undergo a spontaneous fermentation, in which lactic acid bacteria and yeasts play a major role [32]. The natural style is characterised by its long duration. In this work, the effect of the natural-style processing on the hydrolytic and oxidative degradation level of the lipid fraction was evaluated.

Characteristics of olives and quality indices of lipid fraction

Weight, moisture and oil content of table olives obtained during the processing period are given in Table I. The oil content in the olive depends on the cultivar and the ripening stage [3]. The olives of our variety at harvest had an oil content of 44.61%/DM (about 25%/FM).It was slightly higher than those noted for the Natural black olives of three Italian cultivars (*Bella dicerignola, Termite di Bitetto* and *Peranzana)* which oil content was 37.50, 37.38 and 41.07%/DM, respectively [19]. During the processing, the oil content remained unchanged or decreased slightly and reached a value of 43.86% after 150 days.

The results of the quality parameters (acidity, peroxide value and coefficients of specific extinction K_{232} and K_{270}) of the oil of the fruits are shown in Table I. All the values were lower than the limits set by EU Regulation (1991) [21] for the extra virgin olive oil category.

The acidity of the oils (% as oleic acid) increased significantly ($P < 0.05$) from 0.178% at harvest to 0.34% after 60 days, to 0.38% after 120 days and remained almost constant after 150 days of processing. This increase could be explained by the hydrolysis of triglycerides by the lipolytic enzymes of olives and/ or microorganisms in the brine solution [33].Our results were much more consistent with those of López-López et al. [12] who studied the effect of green Spanish-style processing (*Manzanilla and Hojiblanca*) than those obtained on black-ripe olives of Italian varieties (*Bella di Cerignola, Peranzana, Termite di Bitetto*) processed by natural-style [19], whose acidity values oscillated between 2.04 and 2.25 after 8 months of fermentation.

Oxidation of fatty acids is an important reaction that affects the quality of the lipid fraction. The peroxide value, initially low (4.3), increased slightly after 60 days of processing (5.26 meq O_2/kg oil); then remained stable during the rest time (Table I). The observed values were lower to those of Pasqualone et al. [19] who reported a significant increase during the natural-style treatment of Italian varieties collected at the completely black-ripe stage, where the PV varied from 3.6 to 11.7 O_2/kg oil for *Bella di Cerignola* and from 4.4 to 13.1 meq O_2/kg oil for *Peranzana*. A more substantial increase of peroxide value (26 meq O_2/Kg oil) was noted for California-style processed olives [18].

The specific extinctions at 232 nm and 270 nm of oils and fats reflect its oxidation degree. An increase in the K_{232} index was noted after 60 days of (from 1.45 to 1.51), but a slight decrease was observed in the last phase of the fermentation (Table I).

As for the K_{270} index, it showed a significant increase after two months of processing (from 0.10 to 0.12, ($p<0.05$) but remained stable during the remaining time (Table I). Higher levels in K_{270} from oil of green (0.4) and ripe olives by-products (0.3) have been noted [34]. The values obtained for the quality parameters (acidity, peroxide value, K232 and K270) of the lipid fraction during fermentation were in accordance with the standards for extra virgin olive oils ((Implementing Regulation (EU) 2013) [35]. These results showed that the natural process did not affect the lipid fraction too much when it initially has good quality characteristics.

Fatty acids composition

The Fatty Acids composition is shown in Table II. Oleic acid (C18:1) is the main fatty acid (75.90% of the total) in raw olives, followed by C18:2 (9.89%) and C16:0 (9.09%). Our results showed that the different

Table I - Evolution of moisture, total lipid and quality parameters during the natural-style processing Means ± standard deviations (n = 3).

Time (days)	0 (At harvest)	60	120	150
Moisture (%)	43.86 ± 0.53	42.75 ± 0.67	44.69 ± 0.33	43.72 ± 0.04
Neutral total fat (% of DM)	44.61 ± 1.45	42.98 ± 2.19	44.34 ± 1.18	43.86 ± 1.33
Acidity (%)	0.178± 0.031[a]	0.339 ± 0.08 [b]	0.339 ± 0.06 [b]	0.375 ± 0.05 [b]
Peroxide index (meq O_2/kg oil)	4.333 ± 0.29[a]	5.166 ± 0.29 [b]	5.267 ± 0.29 [b]	5.166 ± 0.29 [b]
K_{232} (nm)	1.467 ± 0.04[a]	1.513 ± 0.004 [b]	1.567 ± 0.005 [c]	1.503 ± 0.008 [b]
K_{270} (nm)	0.100 ± 0.001[a]	0.119 ± 0.007 [b]	0.115 ± 0,001 [b]	0.121 ± 0.008 [b]

Different letters in rows indicate significant differences at P <0.05
The results are arranged in ascending order; a < b < c < d

fatty acids undergo very slight variations during processing. The same trend was mentioned by many authors [11, 13, 18, 36].

The stability of the fatty acids of olives prepared by natural style processing could be linked to their structures, where the monounsaturated oleic acid was the major part, and to the protective action of antioxidants. Our variety contains a high content of oleic acid and tocopherols.

For the *trans*-fatty acids including *trans*-linoleic acid (C18:2t) and *trans*-linolenic acid (C18:3t) were absolutely absent, while *trans*-linoleic acid (C18:1t) noted lower values (0.02%) than those of the limits required by the Commission Regulation (EU); ≤0.05 (Commission Implementing Regulation (EU) 2013) [35].

The preservation of the essential components of fatty acids can also be explained by the quality of olive pulp. At this stage of maturation the pulp may be more rigid and this characteristic prevents the diffusion of fatty acids in the external environment of the olive (brine), as well as the absence of alkaline treatment of sodium hydroxide (NaOH) on skin and olive pulp that can cause changes in the susceptible classes of compounds.

Di and Triglycerides composition

The determination of Triglycerides (TG) composition was expressed as the total carbon number (Table II). Each triglyceride may be referred to a well-defined component of fatty acid or to a mixture of different fatty acids.

The triglyceride composition showed a main concentration for C54 with 69.15% of total TG, followed by C52, C50 and C56 that mean values of 26.13-3.11 and 1.46%, respectively. The other specific TG: C48, C58, and mostly C60 and C62 registered a very low level. In general, the triolein (OOO) designated as ECN 48 and which contains 54 carbons is the predominant triglyceride in olive oil [37, 38]. This is linked to the fatty acid composition where oleic acid is predominant. C54 can also be OLO - SOO - SLL - POL and LLO, respectively, from the highest concentration to the lowest [38].

The C52 compounds can be represented by the following triglycerides POO – POL – PSL and PLL, characterised by the presence of the linoleic and palmitic fatty acids as in the second row of the total fatty acids. C50 can be represented by the LnPP and PPO; the C56 can include both of OLA and OOA triglycerides in olive oil [37].

The amount of triglycerides C54 remained stable during the process, only a very slight decrease was noted after 150 days (from 69.15 to 68.90%). The same results were observed for *Manzanilla* and *Hojiblanca* olives processed as green Spanish-style [12]. The presence of polar compounds, such diglyceride, is considered as a measurement of the degradation degree of the oil. A significative increase of diglycerides due to the hydrolysis of triglycerides was noted [19]. In this work, as mentioned above the significant increase of acidity, agreed with a slight hydrolysis of triglycerides into diglycerides and fatty acids. The total diglycerides showed a little increase particularly during the first step where its content varied from 1.51 at harvest to 1.60% after 60 days of processing. The di-glycerides were found originally in the oil of fresh olives at harvest as 1, 2-diglyceride that represents 93.06% of total diglycerides. During processing, the amount of 1, 2-diglyceride showed a significant decrease from 93.06% in fresh olives at harvest to 77.17% after 120 days of brining, while 1, 3-diglyceride showed a significant increase from 6.95% to 22.83% of the total. These results indicated that during processing the diglycerides undergo an isomerisation. The origin of diglycerides was related to the processing, which produced 1, 3-diglycerides [37].

Volatile compounds

Two volatile compounds, hexanal and nonanal aldehydes, were evaluated during processing. These two aldehyde metabolites registered a concentration of 9.26 and 36.32 mg/Kg oil respectively in the olive fruits at harvest. These results trended to increase significantly over time, after 60, 120 and 150 days. Hexanal increased from 9.26 oil to 13.16 mg/Kg oil after 60 days, and then to 21.99 mg/Kg oil after

Table II - Evolution of triglyceride (number of carbon), fatty acids and volatile composition the natural-style. Processing (Means ± standard deviations, n = 3).

Time (days)	0 (At harvest)	60	120	150
	Triglyceride (%)			
C 48	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C 50	3.11 ± 0.01	3.15 ± 0.01	3.12 ± 0.05	3.16 ± 0.02
C 52	26.13 ± 0.02 ^{b}	26.21 ± 0.01 ^{c}	26.01 ± 0.03 ^{a}	26.26 ± 0.04 ^{c}
C 54	69.15 ± 0.01 ^{bc}	69.02 ± 0.04 ^{ab}	69.26 ± 0.10 ^{c}	68.90 ± 0.01 ^{a}
C 56	1.46 ± 0.04	1.44 ± 0.03	1.46 ± 0.01	1.51 ± 0.01
C 58	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.13 ± 0.00
C 60	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
C 62	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Total	100.01 ± 0.00	100.01 ± 0.01	100.01 ± 0.00	100.01 ± 0.00
	Diglyceride(%)			
C 34 1:2	12.32 ± 0.55 ^{b}	10.28 ± 0.18 ^{a}	10.25 ± 0.14 ^{a}	10.30 ± 0.08 ^{a}
C 34 1:3	1.04 ± 0.03 ^{a}	3.13 ± 0.23 ^{b}	3.09 ± 0.04 ^{b}	3.25 ± 0.10 ^{b}
C 36 1:2	80.74 ± 1.63 ^{b}	67.80 ± 0.14 ^{a}	67.92 ± 0.18 ^{a}	66.88 ± 0.60 ^{a}
C 36 1:3	5.91 ± 1.10 ^{a}	18.80 ± 0.56 ^{b}	18.75 ± 0.01 ^{b}	19.58 ± 0.42 ^{b}
Total	100.01 ± 0.01	100.00 ± 0.00	100.01 ± 0.01	100.00 ± 0.00
Amount Diglyceride 1:2	93.06 ± 1.08 ^{b}	78.08 ± 0.33 ^{a}	78.17 ± 0.04 ^{a}	77.17 ± 0.52 ^{a}
Amount Diglyceride 1:3	6.95 ± 1.07 ^{a}	21.92 ± 0.33 ^{b}	21.84 ± 0.03 ^{b}	22.83 ± 0.52 ^{b}
Total Diglyceride	1.51 ± 0.04	1.60 ± 0.04	1.57 ± 0.02	1.57 ± 0.01
	Fatty acids (%)			
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
Totale	100.00 ± 0.01	100.01 ± 0.00	100.00 ± 0.00	100.00 ± 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
Somme des trans	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
	Volatile components (mg/Kg)			
Hexanal (mg/Kg)	9.26 ± 0.18 ^{a}	13.16 ± 0.25 ^{b}	21.99 ± 0.66 ^{d}	16.69 ± 0.33 ^{c}
Nonanal (mg/Kg)	36.32 ± 1.16 ^{a}	45.20 ± 0.78 ^{b}	42.66 ± 0.96 ^{b}	62.48 ± 2.91 ^{c}
Total	45.58 ± 1.34 ^{a}	58.36 ± 1.03 ^{b}	64.65 ± 1.63 ^{c}	79.17 ± 3.24 ^{d}

Different letters in rows indicate significant differences at P <0.05
The results are arranged in ascending order; a < b < c < d

120 days but decreased to 16.69 mg/Kg oil after 150 days (Table II). The nonanal showed a marked increase; values of 45.20, 42.66 and 62.48 mg/kg were noted after 60, 120 days and 150 days of processing respectively. In general, the total volatile compounds had a significant increase trend (P <0.05) after each step of natural processing in brine.

Many studies carried out the contents of volatile compounds and studied the processing effect on the variation of these metabolites of table olives flesh [14, 15]. A meaningful increase over time of some volatile compounds (acids and alcohols) was observed in Greek-style processing flesh of table olive samples [15]. There are clear different biogenesis pathways of volatile compounds between table olives and olive oil. In this study, the evaluation of volatile compound was studied in the oil fraction. As known, C6 and C5 aldehydes and alcohols, and their corresponding esters, are the main volatile compounds in olive oils that are produced by the lipoxygenase pathway of polyunsaturated fatty acids [15].

The increase of Hexanal that is known to have green

Table III - Evolution of Tocopherols (mg/Kg) of oil during the natural-style processing(Means ± standard deviations, n = 3).

Time (days)	0 (At harvest)	60	120	150
Δ-Tocopherol	0.39 ± 0.03	0.45 ± 0.07	0.27 ± 0.01	0.42 ± 0.13
γ-Tocopherol	4.21 ± 0.37	4.34 ± 0.38	4.47 ± 0.23	4.53 ± 0.1
β-Tocopherol	1.48 ± 0.14	1.46 ± 0.12	1.43 ± 0.14	1.41 ± 0.05
α-Tocopherol	133.75 ± 2.97 [b]	131.84 ± 2.52[b]	122.83 ± 0.45 [a]	122.44 ± 0.64 [a]
Total Tocopherol	139.82 ± 3.45 [b]	138.09 ± 2.71 [b]	128.99 ± 0.37 [a]	128.80 ± 0.66 [a]

Different letters in rows indicate significant differences at P <0.05
The results are arranged in ascending order; a < b < c < d

and sweet odours, having a positive effect on the aroma, this could be explained by the degradation of 13-hydroperoxides by the hydroperoxide-lyase (HPL) enzyme or formed by oxidation of linoleic acid.
Nonanal is one of the major compounds formed in oxidized olive. The hexanal/nonanal ratio indicates the oxidation status of olive oils [39]. It increased slightly from 0.25 to 0.29 after 60 days, and then decreased during the last phase of fermentation, indicating a moderate oxidation.
Volatile compounds in olive oil are mainly produced by the oxidation of fatty acids through the lipoxygenase pathway, whereas chemical oxidation and exogenous enzymes, usually from microbial activity, are associated with sensory defects [40].

Tocopherols

Tocopherols are related to the nutritional benefits of table olives including oil fraction. Moreover, they defend the body against free radical attacks by protecting polyunsaturated fatty acids. As shown in Table III, the major isomer was α-tocopherol with 133.75 mg/Kg oil of fresh olives (95.65% of the total tocopherols). Gamma, Beta and, Delta tocopherols were present at very low level of 4.21, 1.48 and 0.39, respectively.
Processing caused a significant (P <0.05) decrease in the content of α-tocopherol (8.5%) after 150 days while it has no effect on other isomers. The α-tocopherol content of our variety is higher than the values reported for the 30 samples of processed olives from the Italian market which vary between 25 and 90 mg / kg [41].
Sakouhi et al. [13] evaluated the changes of α-tocopherol of three varieties during both ripening and processing, according to the Spanish style, they noted that α-tocopherol amounts increased with ripening and decreased during processing. The decrease was more evident in the black stage than in the green one. Our results are high compared to the green and cherry stage of the Tunisian table olives under study (Meski, Sayali and Picholine) [13].

Phenolic compounds

In natural style, the bitterness of oleuropein, ligstroside, and related phenolic compounds can be reduced by their diffusion from the pulp into the brine which requires a long processing time. Oleuropein hydrolysis carried microbial and endogenous enzymes [42]. The composition of the phenolic fraction of table olives is very complex and can vary qualitatively and quantitatively. In this work, the total phenols content of the oil fraction of fresh olives determined by HPLC was about 664.04 mg/Kg oil. A significant decrease (Table IV) was observed during processing; total losses of 27% were noted. These results follow the same trend of those obtained by colorimetric assay (Table IV). The losses are much lower than those recorded by Pasqualone et al. [19] on black-ripe olives, which varied between 64 and 73% of the total phenols content with a significant varietal influencing effect. Few authors reported the effect of processing on the polyphenols' contents of the oil fraction, Romero et al. [6] noted that processing influences more the concentration of polyphenols of the aqueous fraction, than those of the oil fraction due to the polar nature of these components. The same authors reported a low proportion of phenols in the lipid fraction (about 5 – 10%) to the total phenols of table olives.
The oil fraction of the fresh olives at harvest contained a small amount of Oleuropein 3.64 mg/Kg oil but a high concentration of Oleuropein Derivatives (439.38 64 mg/Kg oil), ligostrosid derivatives (183.44 mg/Kg oil) and hydroxytyrosol (79.35 mg/Kg oil). The small amount of oleuropein could be related to the variety or the action of β-glucosidase that has a role in the production of phenyl-aglycone during the malaxation of olive paste [43].
Concerning the flavonoids, the fresh fruit showed an amount of 7.68 mg/Kg oil distributed between luteolin and apigenin with a high amount of luteolin (6.17mg/Kg oil).
A significant decrease in the concentrations of oleuropein and ligstroside derivatives, lignans, luteolin, apigenin and secoiridoids acid was observed after processing. This decrease can be explained by the hydrolysis of phenolic complexes into a range of compounds by microbial and endogenous enzymes [44, 45] and by the diffusion of phenolic compounds from the fruit to brine [46]. The involvement of endogenous enzymes was suggested such as

Table IV - Evolution of Phenolic compounds and antioxidant activity of the lipid fraction of olives during the natural-style processing (Means ± standard deviations, n = 3)

Time (days)	0 (At harvest)	60	120	150
	Colorimetric determination of polyphenols			
Total polyphenols (mg GAE/Kg)	520.98 ± 3.12^{d}	499.05 ± 0.59^{c}	455.13 ± 0.68^{a}	465.17 ± 2.92^{b}
	HPLC determination of polyphenols (mg/Kg)			
Total Biophenols	664.04 ± 6.90^{c}	560.94 ± 1.22^{c}	516.61 ± 0.62^{b}	490.04 ± 2.44^{a}
Natural total Biophenols	657.23 ± 7.30^{c}	554.10 ± 0.93^{c}	509.03 ± 0.62^{b}	478.53 ± 1.74^{a}
Total aromatic Alcohols	102.41 ± 2.39^{c}	95.67 ± 0.20^{b}	26.36 ± 1.03^{a}	28.22 ± 0.05^{a}
Hydroxytyrosol	79.35 ± 0.78^{d}	74.48 ± 0.14^{c}	15.46 ± 0.10^{a}	16.57 ± 0.08^{b}
Tyrosol	14.08 ± 0.05^{d}	11.19 ± 0.06^{b}	10.90 ± 0.01^{a}	11.65 ± 0.04^{c}
Oleuropein	3.64 ± 0.20^{a}	1.97 ± 0.39^{b}	1.41 ± 0.09^{a}	1.10 ± 0.49^{a}
Oleuropein Derivatives	439.38 ± 12.19^{c}	347.76 ± 1.20^{c}	298.37 ± 0.19^{b}	272.59 ± 0.69^{a}
Ligostrosid Derivatives	183.44 ± 1.71^{b}	185.58 ± 0.46^{b}	183.15 ± 0.70^{b}	174.96 ± 0.04^{a}
Oleocanthal	17.95 ± 0.16	13.26 ± 4.31	16.64 ± 0.47	17.61 ± 0.26
Lignanes	30.33 ± 1,29^{b}	5.79 ± 0.13^{a}	7.05 ± 0.21^{a}	8.12 ± 0.17^{a}
Total phenolic Acids	5.97 ± 0.01^{a}	14.31 ± 0.04^{b}	19.51 ± 0.11^{c}	20.55 ± 0.11^{d}
Total Flavonoids	7.68 ± 0.01^{c}	5.92 ± 0.06^{b}	4.55 ± 0.06^{b}	2.32 ± 1.03^{a}
Luteolin	6.17 ± 0.10^{c}	4.66 ± 0.02^{b}	3.77 ± 0.01^{b}	2.05 ± 0.79^{a}
Apigenin	1.51 ± 0.08^{c}	1.26 ± 0.08^{c}	0.78 ± 0.03^{b}	0.27 ± 0.23^{a}
Oxidised total Biophenols	6.82 ± 0.4^{a}	6.84 ± 0.29^{a}	7.58 ± 0.75^{a}	11.51 ± 0.70^{b}
Oxidised ratio: (Oxidised biophenols/ total biophenols) (%)	0.01 ± 0.00^{a}	1.03 ± 0.04^{b}	1.23 ± 0.41^{b}	1.95 ± 0.11^{c}
Hydrolysis ratio: (Total aromatics alcools/ total biophenols) (%)	15.41 ± 0.04^{d}	14.47 ± 0.01^{c}	4.27 ± 0.07^{a}	4.78 ± 0.01^{b}
Total secoiridoidic acids	87.25 ± 0.47^{b}	79.67 ± 6.36^{b}	45.22 ± 0.02^{a}	43.13 ± 0.01^{a}
Decarboxymethyl elenolic acid	1.97 ± 0.01^{d}	1.67 ± 0.00^{c}	1.31 ± 0.18^{b}	0.86 ± 0.02^{a}
Elenolic acid	85.28 ± 0.46^{d}	78.00 ± 0.71^{c}	43.91 ± 0.01^{b}	42.27 ± 0.04^{a}
Total oxidised secoiridoid acid	0.01 ± 0.00^{a}	0.03 ± 0.01^{a}	0.04 ± 0.17^{a}	0.08 ± 0.02^{b}
Total oxidised decarboxy-methyl-elenolic acid	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{a}	0.06 ± 0.02^{a}
Epoxy elenolic acid	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00

Different letters in rows indicate significant differences at P <0.05
The results are arranged in ascending order; a < b < c < d

esterase and β-glucosidase in olive debittering during the first month of brining [47], thereafter the hydrolysis of this polyphenol can be achieved by the action of the exogenous hydrolases excreted by the strains of lactic acid bacteria [44, 45]. The acidic conditions of the brine can also favour the chemical hydrolysis of oleuropein [48]. Our results showed a significant increase of oleanolic acid, a nonphenolic compound that is linked to many phenolic compounds. Other phenomena, such as chemical and enzymatic oxidation, may contribute to the decrease of phenolic compounds. Indeed, an increase of the total oxidised biophenols concentration was noted during fermentation, from 6.82 mg/Kg oil at harvest to 11.51 mg/Kg oil after 150 days. This trend was clearly shown by the evolution of the ratio (oxidised biophenols/total biophenols) that increases from 0 at harvest to 1.95% of total biophenols at the end of processing.

Antioxidant activity

One of the important aspects to study the antioxidants has been the measurement of antioxidant activity by the measure of antiradical activity of the oil against the radical DPPH. Figure 1 showed the antioxidant capacity against radical DPPH of oil fraction and its methanolic extracts. Results indicated that the methanolic extracts exhibited a high antioxidant potential at harvest stage (raw sample), with (277.04 mg GAE/Kg oil), but after processing in brine for 60 days the antioxidant capacity decreased to 210.45 mg GAE/Kg oil, the values decreased significantly to 186.87 mg GAE/Kg oil at the end of processing. The same trend was observed for the antiradical capacity of the oils, but the variation was lower. The fresh fruit oil has an antioxidant capacity of 77.24 mg GAE/kg of oil, which decreased slightly but significantly during the natural processing to 75.35 mg GAE/kg oil. Previous studies showed that the antioxidant capacity of olives is probably related to the polyphenol content, including hydroxytyrol and tyrosol [8, 49]. Moreover, the same phenomenon is observed in virgin olive oil [50]. In our study, the antioxidant capacity of both methanolic extract and

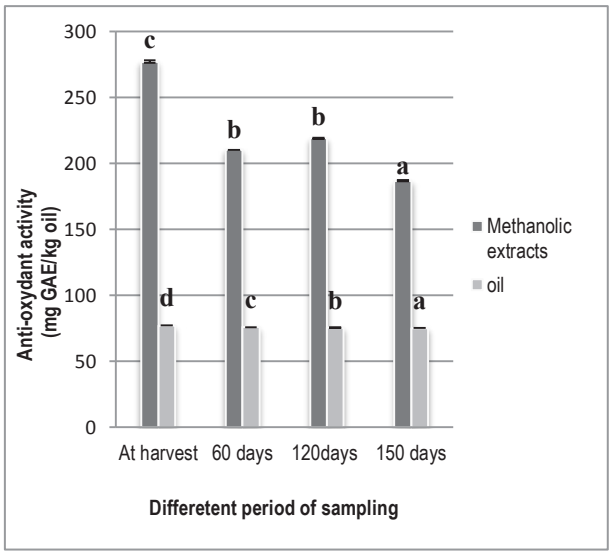

Figure 1 - Antiradical activity against DPPH of oil and its methanolic extracts of different samples. Different letters indicate significant differences at P <0.05
The results are arranged in ascending order; a < b < c < d

the oil of table olives from the Sigoise turning colour decreases with the loss of polyphenol contents. The same results were observed for Tunisian table olives [36] prepared by natural process (in brine) with a strong positive correlation between the total equivalent antioxidant capacity and the total phenols contents. On the other hand, cultivars and ripening stage influence the polyphenol content, hence the antioxidant capacity. As approved by Sousa et al. [51] green olive fruits possess higher antioxidant potential than black olives. The cited works were observed for the aqueous fraction of table olives, and this is the first time that the oil fraction of olives was studied to evaluate its antioxidant capacity.
As shown in Figure 1, the methanolic extract exerts a better activity than the fruit oils. Processing induced a decrease of the antioxidant capacity due to the loss of phenolic compounds during fermentation. The antioxidant activity of the oil fraction of olives against DPPH was less affected by the process than those of its methanolic extracts.

4. CONCLUSION

In conclusion, the results evidenced a significant effect of natural processing on the degradation of the lipid fraction of table olives. The quality parameters (acidity, PV, $K_{232,}$ K_{270} registered a significant increase). However, these indices were always below the limit established for EVOO. Processing did not cause any systematic effect on fatty acids, triglycerides and total diglycerides, whereas, 1, 2 diglycerides decrease by isomerisation to 1, 3 diglycerides. Volatile compounds recorded a slight increase due to the oxidative degradation of polyunsaturated fatty acids. The most identified tocopherol was α-tocopherol, its amount decreased slightly during processing and reached 122.44 mg/Kg oil, which is a good dose to protect fatty acids. The phenolic compounds undergo a reduction in their concentration and a slight oxidation during processing. As a result, a reduction in the antioxidant capacity of the methanolic extracts of the lipid fraction was observed.
Globally, this study showed a limited degradation of lipid fraction with processing, *Sigoise* turning colour table olives elaborated by natural style can be considered as good functional food that could keep the maximum benefits of the components. Its production is a simple, natural process that does not use chemicals.

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