



Effect of Spanish style processing on the phenolic compounds and antioxidant activity of Algerian green table olives

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SUMMARY: The study was carried out on seven Algerian olive cultivars to report the effect of Spanish style processing on individual and total phenolic compounds and the changes that occur in antioxidant capacity. The results indicate that the treatment leads to losses in phenolic contents which are cultivar dependent. *Sigoise* is the least affected variety (12.25%) and *Azzeradj* from Seddouk the most affected one (94.80%). The phenolic profile shows drastic changes after processing. Hydroxytyrosol is dominant in processed olives (14.42–545.42 mg·100 g⁻¹) while oleuropein is the major phenolic compound in fresh olives (994.27 mg·100 g⁻¹). As a consequence to the loss in phenolic content, substantial reductions in the antioxidant activities of the extracts are noted. They are estimated to be 13.12–92.75% in scavenging activity against the DPPH radical, 37.78–93.98% in reducing capacity, 59.45–97.94% in the hydrogen peroxide radical and 7.26–51.66% in the inhibition bleaching of β -carotene. Among the processed varieties, only *Sigoise* presented a positive value of RACI (relative antioxidant capacity index).

KEYWORDS: Antioxidant activity; Phenolic compounds; RACI; Spanish style processing; Table olives

RESUMEN: Efecto del procesamiento sobre el contenido fenólico y la actividad antioxidante de aceitunas verdes de mesa argelinas. El estudio se llevó a cabo con siete variedades de aceitunas argelinas y se investigó el efecto de la elaboración al estilo español sobre los compuestos fenólicos individuales y totales; así como los cambios que se producen en la capacidad antioxidante. Los resultados obtenidos muestran que el procesamiento conduce a pérdidas en el contenido fenólico, lo cual es dependiente de la variedad. *Sigoise* es la variedad menos afectada (12.25%) y *Azzeradj* de Seddouk la más (94.80%). El perfil fenólico muestra cambios drásticos después de su procesamiento. El hidroxitirosol es el polifenol predominante en aceitunas procesadas (14.42–545.42 mg·100 g⁻¹), mientras que la oleuropeína es el compuesto fenólico mayoritario en las aceitunas frescas (994.27 mg·100 g⁻¹). En consonancia con la pérdida en estos polifenoles, se detectaron reducciones sustanciales de la actividad antioxidante de los extractos. Se estima en 13.12 a 92.75% la actividad de eliminación de radicales DPPH 37.78–93.98% en la reducción de la capacidad, 59.45 a 97.941% en el radical peróxido de hidrógeno y 7.26–51.66% en la inhibición de blanqueo del β -caroteno. Entre las variedades procesadas, sólo *Sigoise* presentó un valor positivo de RACI (Índice Relativo de Capacidad Antioxidante).

PALABRAS CLAVE: Aceitunas de mesa; Actividad antioxidante; Compuestos fenólicos; Estilo español de procesamiento; RACI

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1. INTRODUCTION

Table olives (*Olea europaea*) and olive oil are the main constituents of the Mediterranean diet. According to the statistical data (IOC, 2013), the worldwide production of table olives is estimated at more than 2.3 million tons (2013–2014 season). The Algerian production is on the rise, from 67.600 tonnes (average of 2001–2006) to 139.700 tonnes (2007–2013). With this production, Algeria contributes with 3.8 and 6%, respectively, to world production. Table olives possess important biological proprieties, linked to their predominance in monounsaturated fat contents, and to antioxidant compounds like phenolics and tocopherols which have health benefits (Bianchi, 2003). Phenolic compounds have been shown to beneficially alter lipid composition, platelet and cellular function, as well as reduce oxidative damage and inflammation (Cicerale *et al.*, 2010). Soni *et al.* (2006) reported the action of olive pulp extract on gastrointestinal disorders due to their antibacterial activity.

Several factors are known to affect the qualitative and quantitative phenolic profiles of table olives. These bioactive compounds are closely affected by cultivar (Vinha *et al.*, 2005), degree of maturation (Malheiro *et al.*, 2011), growing conditions (Marsilio *et al.*, 2006), fruit size (Amiot *et al.*, 1990) and processing methods (Sahan *et al.*, 2013).

Olives cannot be consumed directly after harvest due to their extreme bitterness and they must undergo various processes. Three kinds of table olives are of economic importance in the international market: Spanish style green olives in brine, Greek style naturally black olives in brine, and California black ripe olives. Spanish style green olives or “alkali-treated green olives in brine” are the most widely distributed (El Khaloui and Nouri, 2007).

Many studies have been carried out regarding the influence of different processing methods of table olives on the levels of total and single phenolics (Romero *et al.*, 2004; Pereira *et al.*, 2006), but very few of them (Ben Othman *et al.*, 2009) have been aimed at assessing the impact of this processing on the antioxidant capacity of the fruits. However, to our knowledge, no studies have been carried out about changes induced by Spanish style processing on Algerian green table olives. Therefore, this study is undertaken to investigate the evolution of phenolic compounds after Spanish style processing and to evaluate the result that those changes have on the antioxidant capacity of the finished product in order to determine the effect of this kind of processing on Algerian olive cultivars.

2. MATERIAL AND METHODS

2.1. Plant material

Olive fruits of seven Algerian cultivars: *Azzeradj* from Seddouk, *Gordal*, *Sevilla*, *Sigoise*, *Taffahi*, *Bouchouk* and *Azzeradj* from Tazmalt were harvested

in October, 2012, at the green maturation stage. Four trees were selected and tagged and the olive fruits were hand-picked from different parts of the olive tree. After sorting and sizing, three kilograms of olives per variety were used.

2.2. Processing

Olives were debittered in an alkali solution ($15 \text{ g}\cdot\text{L}^{-1}$) during 8 to 12 h until the lye had penetrated two thirds of the pulp. Three washing waters are more than sufficient (twice for 4 h and once for 12 h) to eliminate excess alkali. Then, the olives were fermented in brine (8% NaCl) where a lactic fermentation reduces the pH to 4.5.

Samples of fresh and processed olives were freeze-dried at $-58 \text{ }^\circ\text{C}$ (Christ, Alpha 1–4 LD plus, Osterode am Harz, Germany), ground in electric blender (IKA model A 11 B, Staufen, Germany) and stored at $-18 \text{ }^\circ\text{C}$ until analysis.

2.3. Analysis of phenolic compounds

2.3.1. Extraction

Phenolic compounds were extracted according to Mc Donald *et al.* (2001). Freeze dried olive pulps (5 g) were homogenized in 25 mL of *methanol/water* (80:20, v/v). The residue was extracted twice; extracts were combined, and washed with hexane. The extracts were filtered, and then kept cold until analysis.

2.3.2. Total phenolic compounds

The total phenolic content of the extracts was determined with Folin Ciocalteu reagent according to Borzello *et al.* (2000). Total phenol values were expressed as mg gallic acid equivalents per 100 g Dry Weight ($\text{mg GAEq}\cdot 100 \text{ g}^{-1} \text{ DW}$).

2.3.3. HPLC separation and identification of phenolic compounds

The presence and amount of phenolic compounds in the olive extracts were studied by reversed phase HPLC analysis using a binary gradient elution. The analysis was performed by reversed phase HPLC on a LC-10ADVP Shimadzu (Milan, Italy) liquid chromatography equipped with an SPD M10AVP diode array detector (Shimadzu). The chromatographic separation was achieved on a Spherisorb S5 ODS-3 (250 mm \times 4.6 mm i.d.) reversed-phase column (Phenomenex, Macclesfield, UK). The solvent system used was a gradient of solvent A (water: trifluoroacetic acid, 97:3, v/v), and solvent B (acetonitrile: methanol, 80:20, v/v). A step gradient from 5% to 98% B (45 min) was applied at a flow rate of $1 \text{ mL}\cdot\text{min}^{-1}$. Peak quantification was carried out at 279 nm. The main phenolic compounds were identified by comparison

with relative retention times of pure compounds, when available, or by comparing the relative elution order and UV spectra with those reported in the literature (Brenes *et al.*, 2000; Rovellini and Cortesi, 2002). The identity of each peak was confirmed by LC-MS, performed on an LC-10AD VP Shimadzu (Milan, Italy) liquid chromatograph on-line with an LCMS-2010EV Shimadzu (Milan, Italy) mass spectrometer, equipped with an electrospray ionization (ESI) interface. A Discovery HS C18 column (5 μm , 150 mm \times 2.1 mm i.d., Supelco, St. Louis, MO, USA), at a flow rate of 0.35 mL \cdot min $^{-1}$, was used. The solvent system used was a gradient of solvent A (water+formic acid 0.25%), and solvent B (methanol+formic acid 0.25%), with a step gradient from 5% to 55% B (45 min). The ESI mass spectra (m/z 60–900) in the negative ion mode were obtained under the following conditions: interface voltage 4 kV; nebulizer gas flow 1.5 L \cdot min $^{-1}$; block heater temperature 250 °C; curved desolvation line temperature and voltage of 300 °C and –5 V, respectively; Q-Array voltage 0 V DC and 150 V RF; detector voltage 1.5 kV. Some operating parameters (interface voltage and Q-Array voltage) were then modified in order to obtain a moderate fragmentation of the de-protonated molecular ions: an interface voltage of 5 kV and a Q-Array voltage of –50 V DC and 150 V RF were used (Savarese *et al.*, 2007).

2.4. Antioxidant activity

2.4.1. Reducing power

The Ferric Reducing Power of the extracts was measured as reported by Zhan *et al.* (2006) using ferric chloride. The absorbance was then measured at 700 nm and the reducing power was expressed as mg quercetin, butylated hydroxyanisole and butylated hydroxytoluene equivalents per 100 g Dry Weight (mg QE, BHAEq and BHTEq \cdot 100 g $^{-1}$ DW).

2.4.2. DPPH free radical scavenging activity

The procedure reported by Boskou *et al.*, (2006) was adapted. An aliquot of the appropriate dilution of the extract (0.5 mL) was added to a 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) solution (2 mL) and kept in the dark for 30 min. The absorbance was measured at 515 nm and the antiradical activity was expressed as mg quercetin and trolox equivalents per 100 g Dry Weight (mgQEeq andTEeq \cdot 100 g $^{-1}$ DW).

2.4.3. Hydrogen peroxide radical scavenging assay

The hydrogen peroxide radical scavenging activity was determined according to Hemalatha *et al.* (2013), the absorbance was measured at 230 nm after 10 min of incubation at 37 °C. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged H}_2\text{O}_2 = (A_C - A_T) / A_C * 100$$

Where: A_C is the absorbance of the control and A_T is the absorbance of the test.

2.4.4. β -Carotene bleaching assay

The effect of extracts on the β -carotene- linoleic acid emulsion was determined by applying the method reported by Nsimba *et al.* (2008). The absorbance of the tested samples was repeatedly measured every 15 min at 470 nm. The total antioxidant activity was calculated based on the following equation:

$$\% AA = [1 - \frac{A_0 - A_t}{A_{00} - A_{0t}}] * 100$$

Where: AA% is antioxidant activity, A_0 and A_t ; emulsion absorbance at $t=0$ and after incubation time t ($t=105$ min), A_{00} and A_{0t} : absorbance values for negative control at $t=0$ and after the same incubation time.

2.5. Statistical analysis

All experiments were conducted in triplicate and a statistical analysis was done using Statistica 5.5 with the analysis of variance (ANOVA/MANOVA) to determine the significant differences at a level of confidence of ($P < 0.05$). Correlation coefficients were calculated using the Pearson coefficient.

3. RESULTS AND DISCUSSION

3.1. Phenolic compounds

3.1.1. Total phenolic content

Phenolic contents (Table 1) differ significantly ($p < 0.05$) among the investigated olive cultivars, and between fresh and processed olives for the same cultivar. Their content are higher in fresh olives (346 mg \cdot 100 g $^{-1}$ in *Bouchouk* to 2406 mg \cdot 100 g $^{-1}$ in *Azzeradj* from Seddouk), than in processed ones (124 mg \cdot 100 g $^{-1}$ in *Azzeradj* from Seddouk to 1688 mg \cdot 100 g $^{-1}$ in *Sigoise*).

The results obtained for fresh olives are similar to those obtained for Portuguese cultivars (Pereira *et al.* 2006), Tunisian cultivars (Ben Othman *et al.* 2009), and Italian cultivars Piscopo *et al.* (2014). However, processed olives contained higher amounts of phenolic compounds than those studied by Blekas *et al.* (2002) and similar contents to those reported by Ben Othman *et al.* (2009).

A drastic decrease in phenolic content was noted after processing. Losses in those compounds differ largely among the cultivars, from 12.25% (*Sigoise*)

TABLE 1. Total and simple phenolic contents of fresh and processed table olives

	Azz Sed	Gordal	Sevilla	Sigoise	Teffahi	Bouchouk	Azz Taz
Total phenolic content (mg GAEq/100 g DW)	F: 2406.87±15.1 ^m P: 124.93±3.92 ^a	2141.3±8.97 ^k 545.06±4.23 ^e	1950.50±12.8 ^j 727.15±7.84 ^f	1923.85±1.02 ^l 1688±2.55 ⁱ	1651.62±9.18 ^h 475.85±11.65 ^d	346.50±4.86 ^c 283.43±1.41 ^b	1220.25±14.20 ^g 359.05±1.18 ^c
Simple phenolic content (mg/100 g DW)							
Oleuropein	F: 994.26±26.64 ^g P: 0.00±0.00 ^a	836.42±40.56 ^f 0.00±0.00 ^a	563.66±15.43 ^e 0.00±0.00 ^a	433.03±0.00 ^d 184.29±49.27 ^d	270.16±81.29 ^c 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	427.03±24.71 ^d 0.00±0.00 ^a
Oleuropein-der	F: 375.63±0.23 ^f P: 0.00±0.00 ^a	164.62±5.53 ^d 233.23±13.68 ^e	164.51±11.34 ^d 129.15±54.85 ^{cd}	167.79±0.00 ^d 124.43±24.02 ^{cd}	105.45±21.04 ^{b,c} 79.84±11.19 ^{b,c}	78.75±10.93 ^{b,c} 56.65±6.21 ^b	170.43±9.72 ^d 0.00±0.00 ^a
Hydroxytyrosol	F: 255±72.15 ^g P: 105.97±12.2 ^{bc}	159.39±16.05 ^c 45.68±1.49 ^a	168.98±5.01 ^c 545.42±13.24 ^f	445.5±0.00 ^f 98.80±19.8 ^b	29.79±0.00 ^a 14.49±1.49 ^a	23.21±0.68 ^a 100.60±1.65 ^b	135.342±11.37 ^b 38.22±0.00 ^a
Hydroxytyrosol-Glu	F: 141.64 ^d ±7.06 P: 0.00±0.00	257.97±31.75 ^e 80.23±5.18 ^c	172.93±16.54 ^{de} 81.03±7.90 ^c	99.00±0.00 ^c 71.10±9.58 ^{bc}	38.51±0.00 ^{ab,bc} 14.66±2.85 ^{ab}	61.35±1.77 ^{bc} 16.72±1.56 ^{ab}	174.81±23.86 ^c 64.52±0.00 ^{bc}
Verbascoside	F: 391.08±5.50 ^d P: 0.00±0.00 ^a	200.84±12.32 ^c 0.00±0.00 ^a	393.30±6.63 ^d 0.00±0.00 ^a	407.54±0.00 ^d 131.58±18.34 ^b	138.57±0.00 ^b 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	207.03±20.06 ^c 0.00±0.00 ^a
Verbascosid-der	F: 215.57±42.27 ^{bc} P: 0.00±0.00 ^a	151.44±14.17 ^{bc} 0.00±0.00 ^a	270.12±3.35 ^c 0.00±0.00 ^a	306.47±0.00 ^d 100.57±25.48 ^{ab}	106.54±14.57 ^{ab} 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	104.40±6.37 ^{ab} 0.00±0.00 ^a
Quercetine 3-Galactosid	F: 205.29±0.61 ^c P: 0.00±0.00 ^a	356.65±26.98 ^e 0.00±0.00 ^a	188.22±19.59 ^c 0.00±0.00 ^a	256.77±0.00 ^d 120.92±32.78 ^c	109.47±16.68 ^b 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	187.73±12.76 ^c 0.00±0.00 ^a
Tyrosol	F: 53.23±0.55 ^c P: 37.27±0.73 ^c	84.08±2.10 ^g 43.65±1.09 ^d	80.13±1.94 ^g 106.49±0.26 ^b	76.60±0.00 ^f 35.84±8.76 ^c	25.44±0.00 ^b 24.38±0.00 ^b	22.26±0.16 ^b 27.60±4.23 ^b	48.72±0.47 ^c 24.35±0.00 ^b
Ligstroside	F: 334.89±86.71 ^e P: 0.00±0.00 ^a	164.39±3.95 ^d 0.00±0.00 ^a	140.06±3.90 ^{cd} 0.00±0.00 ^a	70.31±0.00 ^{ab} 52.68±16.76 ^{ab}	86.02±3.91 ^{bc} 51.10±0.00 ^{ab}	65.30±8.64 ^{ab} 0.00±0.00 ^a	114.52±3.79 ^d 0.00±0.00 ^a
Acid benzoic/ac cinnamic-der	F: 0.00±0.00 ^a P: 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a
Benzoic acid-der	F: 0.00±0.00 ^a P: 0.00±0.00 ^a	476.08±7.01 ^f 0.00±0.00 ^a	195.75±37.6 ^c 295.45±34.64 ^{de}	471.13±0.00 ^f 258.66±51.56 ^d	0.00±0.00 ^a 101.69±20.31 ^b	51.26±2.35 ^c 195.80±3.32 ^c	133.94±0.00 ^c 0.00±0.00 ^a
Hydroxytyrosol-elenolic acid	F: 295.68±17.74 ^f P: 0.00±0.00 ^a	98.90±2.35 ^c 0.00±0.00 ^a	159.82±5.79 ^e 0.00±0.00 ^a	121.96±0.00 ^d 74.102±21.88 ^b	0.00±0.00 ^a 0.00±0.00 ^a	0.00±0.00 ^a 0.00±0.00 ^a	110.84±7.71 ^{cd} 0.00±0.00 ^a
Acid caffeic-der	F: 133.57±10.95 ^{bc} P: 35.37±3.6 ^a	221.95±33.78 ^e 197.86±22.65 ^{cd,de}	212.91±16.13 ^{de} 141.38±3.27 ^{bc}	146.25±0.00 ^{bc} 156.4±59.13 ^{bc,cd}	142.95±35.82 ^{b,c} 0.00±0.00 ^a	107.26±1.80 ^b 56.42±3.08 ^a	136.23±27.20 ^{bc} 0.00±0.00 ^a
Flavonoids	F: 231.51±34.72 ^g P: 0.00±0.00 ^a	215.93±8.10 ^g 0.00±0.00 ^a	103.54±16.1 ^{cd} 0.00±0.00 ^a	149.81±41.63 ^{de} 142.72±0.00 ^{de}	137.74±33.06 ^{de} 47.09±10.49 ^{ab}	0.00±0.00 ^a 0.00±0.00 ^a	185.90±4.38 ^{ef} 76.93±0.00 ^{bc}

F: fresh olives; Azz Sed: *Azzeratji* from Seddouk; Der: derivative; P: Processed olives; Azz Taz: *Azzeratji* from Tazmalt. Results represent averages of three replicates. Different letters in the same line, and between fresh and processed olives for the same cultivar indicate significant difference ($p < 0.05$).

to 94.80% (*Azzeradj* from *Seddouk*). This trend confirms that processing influence phenolic behavior differently between cultivars according to their phenolic profile. In fact, the diffusion of phenols from olive pulp to brine depends on cultivar characteristics, fruit skin, permeability, type of phenols present in the olives and their ability to diffuse outside the fruit (Kiai and Hafidi, 2014).

3.1.2. HPLC identification of phenolic compounds

The HPLC analysis of phenolic compounds (Table 1) showed that olive flesh revealed different phenolic compound compositions for the seven analyzed cultivars with a drastic difference between fresh and processed olives.

The main phenolic compound detected in fresh olives was oleuropein; its proportion ranged from 25.43% (*Sevilla*) to 39.10% (*Gordal*). The oleuropein level was variety dependant and *Azzeradj* from *Seddouk* presented the highest amount (994.27 mg·100 g⁻¹), while in *Bouchouk* this compound was not detected. These significant differences could be explained by the cultivated variety, cultivars with large fruit size (data not shown) have higher amounts of oleuropein (*Azzeradj* from *Seddouk* and *Gordal*) than the small size ones (*Sigoise* and *Bouchouk*) as reported previously by Amiot *et al.* (1990). The analyzed cultivars showed higher amounts of oleuropein compared to Tunisian cultivars (Ben Othman *et al.*, 2009) and Italian cultivars (Piscopo *et al.*, 2014). The results confirmed the effect of cultivar and geographical origin on the phenolic content of olives.

Verbascoside was the second most abundant phenolic compound; its values ranged from 391.08 to 407.548 mg·100 g⁻¹ for *Azzeradj* from *Seddouk* and *Sigoise*, respectively. The amounts of ligstroside vary between 65.30 mg·100 g⁻¹ (*Bouchouk*) and 334.89 mg·100 g⁻¹ (*Azzeradj* from *Seddouk*).

Similarly to the evolution of oleuropeine, verbascoside and ligstroside contents showed a decline in processed olives; their contents were under quantification limits in processed olives for the majority of cultivars, excepted for *Sigoise*. The results obtained confirm previous data (Boskou *et al.*, 2006; Kiai and Hafidi, 2014) which reported a decrease in oleuropein and verbascoside contents, similarly to an increase in hydroxytyrosol. Processing according to Spanish style causes hydrolysis of phenol complexes due to NaOH treatment, leading to the liberation of simple phenols. Tyrosol showed a slight decrease as well after processing. This observation was not in agreement with the findings of Sahan *et al.* (2013) who observed an increase in the tyrosol content arising from ligstroside hydrolysis. In addition, fresh olives are rich in quercetin-3 galactoside (109.47 mg·100 g⁻¹ in *Teffahi* to 356.65 mg·100 g⁻¹ in *Gordal*). These values are much higher than the amount reported by Piscopo *et al.* (2014) in

Italian cultivar; with the exception of *Bouchouk* (0.00 mg·100 g⁻¹).

In processed olives, the first major phenolic compound was hydroxytyrosol. The highest content was recorded for *Sevilla* (545.42 mg·100 g⁻¹ DW equivalent to 109 mg·100 g⁻¹ FW), this result is higher than that of Sahan *et al.*, (2013) (26.45 mg·100 g⁻¹ FW). The lowest level was observed in *Teffahi* (14.49 mg·100 g⁻¹). It is known that hydroxytyrosol derives from the hydrolysis of oleuropein and verbascoside. An increase in the content of this compound was observed only in *Sevilla* and *Bouchouk* varieties. In contrast, for other varieties, a decrease was noted. This might be explained by the diffusion of this polar compound to the brine, or its oxidation during de-bittering (Pasqualone *et al.*, 2014).

Caffeic acid, which is derived from the hydrolysis of verbascoside, was not detected in processed olives. Rodriguez *et al.* (2008) demonstrated that phenolic acids as caffeic, p-coumaric and ferulic acids are metabolized by *L-plantarum*, which contain a phenolic acid decarboxylase, to their corresponding vinyl derivatives. Romero *et al.* (2004) showed that simple phenolic compounds are converted by lactic acid bacteria and de-polymerized.

3.4. Antioxidant activity

3.4.1. Reducing power

The ferric reducing power of the phenolic extracts of fresh and processed olives are represented in Table 2 (The three standards used recorded the same level of significance). The fresh olives of *Azzeradj* from *Seddouk* exhibited the strongest reducing capacity (4531.90 mg QEq/100 g; 7784.4 mg BHAEq·100 g⁻¹; 9180.82 mg BHTEq·100 g⁻¹) while *Bouchouk* recorded the lowest one (522.45 mg QEq·100 g⁻¹; 887.40 mg BHAEq·100 g⁻¹; 1058.66 mg BHTEq·100 g⁻¹).

Processing caused a decrease in the reducing capacity of extracts. The values ranged from 283.36 mg QEq·100 g⁻¹, 486.72 mg BHAEq·100 g⁻¹, 574.85 mg BHTEq·100 g⁻¹ (*Azzeradj* from *Seddouk*) to 2316.28 mg QEq·100 g⁻¹, 3978.64 mg BHAEq·100 g⁻¹, 4687.47 mg BHTEq·100 g⁻¹ (*Sigoise*). So that, decreases in reducing power after processing were estimated to 37.78% (*Sigoise*), 52.42% (*Sevilla*), 55.25% (*Azzeradj* from *Tazmalt*), 68.39% (*Teffahi*), 76.09% (*Gordal*) and 93.98% (*Azzeradj* from *Seddouk*).

The results showed that a reduction in reducing power is related to a loss in phenolic content. A significant correlation (r=0.98) (Table 3) was obtained between the two parameters. *Azzeradj* from *Seddouk* recorded the highest phenol content loss (9.80%) and showed the highest reducing capacity loss (93.98%). In contrast, *Sigoise*, in which the phenolic content was more preserved, showed the lowest reducing power reduction (37%). No change is noted in reducing power for

TABLE 2. Antioxidant activities of methanolic extracts of fresh and processed olives

Cultivar	SA DPPH (mg Eq/100g DW)				Reducing Power (mg Eq/ 100g DW)				SA H ₂ O ₂ (%)	IBBC (%)
	Quercetin Eq	Trolox Eq	α -tocopherol Eq	Quercetin Eq	BHA Eq	BHT Eq	BHT Eq	BHT Eq		
<i>Azz Sed F</i>	1972.62±6.71 ^{kl}	5458.53±18.57 ^{kl}	14365.51±48.86 ^{kl}	4531.90±255.67 ⁱ	7784.40±439.15 ⁱ	9180.82±517.93 ⁱ	9180.82±517.93 ⁱ	9180.82±517.93 ⁱ	85.02±0.69 ^g	72.17±3.39 ^{ef}
<i>Azz Sed P</i>	143.00±0.21 ^a	395.71±0.58 ^a	1041.40±1.54 ^a	283.36±0.66 ^a	486.73±1.13 ^a	574.85±1.39 ^a	574.85±1.39 ^a	574.85±1.39 ^a	1.75±0.39 ^a	34.88±2.03 ^a
<i>Gordal F</i>	1853.34±27.34 ^l	5128.46±75.64 ^l	13496.85±199.07 ^l	3748.66±104.43 ^k	6439.02±179.39 ^k	7594.11±211.57 ^k	7594.11±211.57 ^k	7594.11±211.57 ^k	88.89±2.22 ^h	71.69±0.84 ^{ef}
<i>Gordal P</i>	503.64±2.47 ^d	1393.65±6.84 ^b	3667.74±18.01 ^d	928.44±23.08 ^c	1594.77±39.65 ^c	1868.54±47.16 ^c	1868.54±47.16 ^c	1868.54±47.16 ^c	2.49±0.39 ^a	75.14±0.93 ^{ef}
<i>Sevilla F</i>	1453.74±32.30 ⁱ	4022.72±89.39 ⁱ	10586.83±235.26 ⁱ	3063.5±294.56 ⁱ	5262.15±505.96 ⁱ	6206.11±596.72 ⁱ	6206.11±596.72 ⁱ	6206.11±596.72 ⁱ	88.67±9.67 ⁱ	73.95±1.27 ^{ef}
<i>Sevilla P</i>	783.90±1.12 ^f	2169.18±3.09 ^j	5708.74±8.14 ^f	1556.35±0.00 ^e	2673.32±2.62 ^e	3154.86±0.99 ^e	3154.86±0.99 ^e	3154.86±0.99 ^e	24.21±3.51 ^d	45.48±4.15 ^b
<i>Sigoise F</i>	1660.05±44.18 ^k	4593.61±122.26 ^k	12089.25±321.77 ^k	3723.16±100.42 ^j	6395.23±172.49 ^j	7542.47±203.43 ^j	7542.47±203.43 ^j	7542.47±203.43 ^j	68.09±0.65 ^f	81.29±1.86 ^f
<i>Sigoise P</i>	1693.05±24.60 ^j	4684.93±68.08 ^j	12329.58±179.16 ^j	2316.28±12.27 ^g	3978.64±21.07 ^g	4687.18±24.99 ^g	4687.18±24.99 ^g	4687.18±24.99 ^g	21.24±0.93 ^d	75.25±3.47 ^{ef}
<i>Teffahi F</i>	1384.64±9.17 ^h	3831.49±25.37 ^h	10083.55±66.78 ^h	3104.11±1789.52 ^h	5331.90±81.01 ^h	6288.38±95.54 ^h	6288.38±95.54 ^h	6288.38±95.54 ^h	13.90±3.77 ^c	78.36±2.88 ^{ef}
<i>Teffahi P</i>	646.79±14.10 ^e	1789.77±39.03 ^e	4710.23±102.72 ^e	997.018485±8.12 ^d	1712.57±13.94 ^d	2026.75±16.80 ^d	2026.75±16.80 ^d	2026.75±16.80 ^d	12.89±0.26 ^{bc}	62.65±2.43 ^d
<i>Bouchouk F</i>	180.24±2.75 ^a	498.76±7.60 ^a	1312.62±20.01 ^a	522.45±11.34 ^b	897.40±19.48 ^b	1058.39±22.98 ^b	1058.39±22.98 ^b	1058.39±22.98 ^b	10.68±1.30 ^b	61.81±0.25 ^d
<i>Bouchouk P</i>	271.54±3.21 ^b	751.39±8.87 ^b	1977.47±23.36 ^b	576.74±23.8 ^b	990.66±40.88 ^b	1155.19±48.66 ^b	1155.19±48.66 ^b	1155.19±48.66 ^b	4.33±1.69 ^a	53.75±11.10 ^c
<i>Azz Taz F</i>	881.94±16.96 ^g	2440.45±46.93 ^g	6422.68±123.51 ^g	1946.18±45.91 ^f	3342.93±78.86 ^f	3942.62±93.01 ^f	3942.62±93.01 ^f	3942.62±93.01 ^f	19.79±2.30 ^d	75.08±1.52 ^{ef}
<i>Azz Taz P</i>	453.27±1.99 ^e	1254.26±5.50 ^e	3300.92±14.48 ^e	863.89±10.54 ^e	1483.90±18.10 ^e	1747.40±21.40 ^e	1747.40±21.40 ^e	1747.40±21.40 ^e	30.45±7.24 ^e	67.90±3.47 ^{de}

F: fresh olives; *Azz Sed*: *Azzeradj* from Seddouk; IBBC: inhibition of bleaching of beta-carotene.P: Processed olives; *Azzj Taz*: *Azzeradj* from Tazmalt.

Results represent average of three replications.

Different letters in the same line, and between fresh and processed olives for the same cultivar indicate significant difference (p<0.05).

TABLE 3. Correlation coefficients for the relationship between antioxidant activities and phenolic content of the extracts

Var	TP	Oleuro	Hydroxy	DPPHQ	DPPH-T	DPPH- α T	RP-Q	RP-BHA	RP-BHT	Querc	Ligst	vebasco	Tyrosol	hydroxy-glu	Ac-hydroxy	B-carot	H ₂ O ₂
Var	1.000																
TP	-0.429	1.000															
Oleuro	-0.488	0.900*	1.000														
Hydroxy	-0.335	0.336	0.261	1.000													
DPPHQ	-0.533*	0.955*	0.858*	0.414	1.000												
DPPH-T	-0.490	0.967*	0.830*	0.357	0.939*	1.000											
DPPH- α T	-0.490	0.967*	0.830*	0.357	0.939*	1.000*	1.000										
RP-Q	-0.444	0.980*	0.905*	0.408	0.979*	0.947*	0.947*	1.000									
RP-BHA	-0.444	0.980*	0.905*	0.408	0.979*	0.947*	0.947*	1.000*	1.000								
RP-BHT	-0.444	0.980*	0.905*	0.408	0.979*	0.947*	0.947*	1.000*	1.000	1.000							
Querc	-0.328	0.866*	0.830*	0.113	0.733*	0.866*	0.787*	0.787*	0.787*	1.000							
Ligst	-0.427	0.786*	0.911*	0.099	0.750*	0.694*	0.786*	0.786*	0.786*	0.689*	1.000						
Verbasco	-0.333	0.850*	0.754*	0.118	0.714*	0.821*	0.752*	0.752*	0.752*	0.828*	0.749*	1.000					
Tyrosol	-0.473	0.451	0.371	0.595*	0.392	0.486	0.390	0.390	0.390	0.522	0.229	0.474	1.000				
Hydroxy-Glu	-0.265	0.704*	0.779*	0.211	0.546*	0.613*	0.638*	0.638*	0.638*	0.819*	0.644*	0.671*	0.631*	1.000			
Ac-Hydroxy	0.051	-0.422	-0.470	0.085	-0.358	-0.434	-0.378	-0.378	-0.378	-0.508	-0.459	-0.494	0.104	-0.153	1.000		
B-Carot	0.096	0.678*	0.510	-0.105	0.574*	0.587*	0.637*	0.637*	0.636*	0.560*	0.434	0.563*	-0.14	0.519	-0.103	1.000	
H ₂ O ₂	-0.459	0.824*	0.875*	0.413	0.829*	0.774*	0.838*	0.838*	0.838*	0.703*	0.722*	0.658*	0.460	0.732*	-0.335	0.456	1.000

*Significant linear correlation at p<0.05.

TP: Total phenolic.

DPPH-Q (Q: quercetin eq), DPPH-T (T:trolox eq), DPPH- α T (α -tocopherol eq).

RP=reducing power, RP-Q (quercetin eq), RP-BHA (BHA eq); RP-BHT (BHT eq).

Bouchouk, which is probably due to its preservation of phenolic compounds.

The potential anti-oxidant index (PAOXI) values of extracts are given in Table 4. Lower PAOXI indicates a lower phenolic content and a better efficiency (Sun and Tanumihardjo, 2007). Our results indicated that all the samples (fresh and processed olives) are efficient because of their PAOXI values of <1. However, the differences noted among PAOXI values might be related to the differences in the phenolic profile of each cultivar. The five varieties processed according to the Spanish style exhibited a better efficiency than their corresponding fresh olives. They are classified as: *Azzeradj* from Tazmalt=>*Azzeradj* from Seddouk>*Sevilla*>*Teffahi*>*Bouchouk*. This indicates that the processing had a positive effect on phenolic profiles (in some cultivars), which permits the transformation of glycoside to aglycone forms, which is more effective.

3.4.2. DPPH radical scavenging activity

The scavenging capacity of the extracts (Table 2) showed no statistically significant difference between *Gordal*, *Azzeradj* from Seddouk and *Sigoise* for fresh olives. These cultivars exhibited the highest scavenging capacities. Differences noted among the other varieties should certainly be related to the differences in the phenolic profiles of cultivars.

The effect of processing on scavenging capacity differs among the cultivars; as a consequence

of the decline in phenolic content, a reduction was noted for six cultivars with remarkable differences in loss percentages: 92.75% (*Azzeradj* from Seddouk), 72.82% (*Gordal*), 53.28% (*Teffahi*), 48.60% (*Azzeradj* from Tazmalt), 46.1% (*Sevilla*) and only 13.12% for *Sigoise*, which was the best efficient extract among processed olives. This in agreement with the results of Sahan *et al.* (2013) for the *Gemlik* cultivar. On the contrary, *Bouchouk* showed an increase (33.57%) in antioxidant activity after processing, which can be attributed to the increase in cinnamic acid derivatives (100%), hydroxytyrosol (76.93%), benzoic acid derivatives (49.96%) and tyrosol (19.35%), confirming that the antioxidant effect does not depend only on the phenolic content but also on the phenolic profile. On the other hand, Brenes and de Castro (1998) claimed that the antioxidant activity of hydroxytyrosol is higher than that of oleuropein. Velkov *et al.* (2007) ranked the phenols of green olives according to their scavenging activity as: dihydrocaffeic acid>hydroxytyrosol>caffeic acid>oleuropein.

According to the Folin Ciocalteu assay, *Gordal* showed a higher phenolic content than *Teffahi*, while it presented the lowest antioxidant capacity; these contradictory results reflect the influence of the nature of phenolic compound on antioxidant activity.

A graphic representation of DPPH inhibition (%) (C (mg·mL⁻¹)) revealed a perfect linearity for all samples (0.993≤r≤0.997) (data not shown) which indicates that the extract scavenging effect on DPPH radical increases with increasing concentrations. The scavenging capacity of fresh olives according to the effective concentrations (EC₅₀) (Table 2) followed the order: *Azzeradj* from Seddouk>*Sevilla*>*Sigoise*>*Gordal*>*Teffahi*>*Azzeradj* from Tazmalt>*Bouchouk*. For the processed olives the order was: *Sigoise*>*Sevilla*>*Gordal*>*Teffahi*>*Azzeradj* from Tazmalt>*Bouchouk*>*Azzeradj* from Seddouk. The results obtained for fresh olives (except for *Bouchouk* and *Azzeradj* from Tazmalt) are in good agreement with those of Arslan and Ozcan (2011) and Malheiro *et al.* (2011). Otherwise, Ilias *et al.* (2011) revealed an effective concentration two times lower for *Sigoise* from Tlemcen; this can be related to differences in composition linked to the geographical origin (Vinha *et al.*, 2005).

The effective concentration (EC₅₀) of extracts exhibited an inverse relationship with phenol contents, showing a significant negative correlation (r=-0.772). Phenolic compounds of olive extracts are good hydrogen donors.

Sigoise processed olives were more effective in scavenging DPPH radical, showing smaller EC₅₀ values (0.38 mg/mL) than those found by Sousa *et al.* (2008) for the Portuguese Alcaparra variety. These results confirm the superiority of *Sigoise* processed cultivar in antioxidant activity, which can be related to its higher quercetine-3 galactosid and caffeic acid contents than other processed

TABLE 4. PAOXI values of extracts corresponding to reducing power, scavenging activity and inhibition bleaching of β-carotene

	RP	SAH ₂ O ₂	IBBC
<i>Azzsed</i>	F: 0.514 ^d	F: 0.286 ^b	F: 0.336 ^g
	P: 0.429 ^a	P: 0.626 ^c	P: 0.035 ^a
<i>Gordal</i>	F: 0.560 ^e	F: 0.237 ^{a,b}	F: 0.299 ^f
	P: 0.597 ^f	P: 2.209 ^f	P: 0.072 ^b
<i>Sevilla</i>	F: 0.594 ^f	F: 0.208 ^a	F: 0.263 ^c
	P: 0.468 ^b	P: 0.317 ^b	P: 0.161 ^c
<i>Sigoise</i>	F: 0.584 ^{e,f}	F: 0.319 ^b	F: 0.267 ^e
	P: 0.805 ^h	P: 0.858 ^d	P: 0.248 ^e
<i>Teffahi</i>	F: 0.527 ^d	F: 1.053 ^e	F: 0.210 ^d
	P: 0.480 ^{b,c}	P: 0.369 ^b	P: 0.076 ^b
<i>Bouchouk</i>	F: 0.648 ^g	F: 0.323 ^b	F: 0.055 ^{a,b}
	P: 0.505 ^{c,d}	P: 0.575 ^c	P: 0.054 ^{a,b}
<i>Azz Taz</i>	F: 0.632 ^g	F: 0.578 ^c	F: 0.161 ^c
	P: 0.419 ^a	P: 0.106 ^a	P: 0.054 ^b

F: fresh olives P: Processed olives.

RP: Reducing Power SAH₂O₂: Scavenging activity against H₂O₂ IBBC: Inhibition Bleaching of β-carotene.

Different letters in the same column indicate significant difference (p<0.05).

olives. The high EC₅₀ values obtained for *Bouchouk* and *Azzeradj* from Seddouk were justified by their polyphenol contents.

3.4.3. Hydrogen peroxide radical scavenging assay (SAH₂O₂)

Inhibition percentages of hydrogen peroxide in fresh and processed green olives (Table 2) at a concentration of 3.571 mg·mL⁻¹ showed significant differences (p<0.05). Fresh olives of the *Gordal* and *Sevilla* varieties exhibited the strongest scavenging activities (88.89 and 88.67%) similar to that of the *Chemlal* cultivar analyzed by Nadour *et al.* (2012) but at a concentration of 0.25 mg·mL⁻¹. *Bouchouk* exerts the lowest one (10.68%).

Losses in scavenging activities after processing were estimated to be 97.94, 97.19, 72.69, 68.8 and 59.45%, respectively, for *Azzeradj* from Seddouk, *Gordal*, *Sevilla*, *Sigoise* and *Bouchouk*. After processing, *Teffahi* recorded statistically the same activity and *Azzeradj* from Tazmalt showed an increase of 35.0%. This may be related to the formation of more effective phenols after processing, and mainly to the generation of phenolic acids (133.94 mg·100 g⁻¹) (hydroxycinnamic and hydroxybenzoic acid derivatives).

The antioxidant activity of flavonoids against H₂O₂ was not significant (r=0.404) (Table 3). However, if we consider separately fresh samples and processed samples, this coefficient was 0.814 and 0.361, respectively. Thus, the loss in this coefficient estimated at 2.25 explains the negative effect of processing by Spanish style on the flavonoid contents of green olives.

Potential antioxidant index (PAOXI) values of the extracts (Table 4) confirmed losses in scavenging activities by the increase of PAOXI values for the cultivars *Sevilla*, *Bouchouk*, *Azzeradj* from seddouk, and *Sigoise*. Although *Gordal* showed a very low efficiency of its phenolic compounds, requiring the implication of more than two phenolic fractions to enrich by one scavenging activity unity. The PAOXI value obtained for *Azzeradj* from Tazmalt is in agreement with the increase in scavenging activity, recording the lowest PAOXI value among the studied cultivars.

3.4.4. β-carotene bleaching assay

The percentages of inhibition bleaching of β-carotene (IBBC) for fresh and processed olive extracts at the concentration of 7.14 mg·mL⁻¹ are given in Table 2. No significant differences (p≤0.05) were noted between fresh and processed olives except for *Azzeradj* from Seddouk and *Sevilla*. This indicates that the phenolic compounds of green olives (fresh and processed) react strongly in an organic medium, probably due to their partition coefficient.

Losses in antioxidant activity after processing varied according to the cultivar: *Azzeradj* from

Seddouk (51.66%), *Sevilla* (38.5%), *Teffahi* (20.04%), *Bouchouk* (13.01%), *Azzerad* from Tazmalt (9.56%), *Sigoise* (7.26%). However, an increase was recorded for *Gordal* which is probably due to the increase in phenolic acids, such as in cinnamic acid derivatives. Skora and Cisowski (2003) postulated that phenolic acids are the weakest inhibitors in lipid peroxidation. Otherwise, contrary to the findings of Han *et al.* (2012), who reported a synergism action between flavonoids and β-carotene accordingly at the water/lipid interfaces, our results show a decline in flavonoid content and similar antioxidant activity after processing. A moderate correlation was established between β-carotene bleaching assay and total phenolics (r=0.67) (Table 3), this means that lipid peroxidation inhibitory activity could be partially correlated to the phenolic content.

The PAOXI values obtained (Table 4) clearly showed that the majority of extracts had better activities in an emulsion medium than in an aqueous one. This may be explained by the “Polar paradox” phenomenon characterized by the accumulation of a polar antioxidant in the oil-water interface, thus protecting the lipids from oxidation (Hayes *et al.*, 2011).

3.4.5. Evaluation of table olive total antioxidant capacity

To get a complete picture of the ranking of the antioxidant capacities of table olives, a relative antioxidant capacity index (RACI) was calculated by integrating the antioxidant capacity values generated from the different tests. RACI is the mean value of standard scores transformed from the initial data generated with different methods (Sun and Tanumihardjo, 2007). Results of the classification of samples (Figure 1) revealed the superiority of the fresh olives of *Azzeradj* from Seddouk in the totality of tests, giving a RACI value of +1.53. The processed olives of the same variety present the lowest RACI value (-1.41). The results showed that among the processed olives, only *Sigoise* denoted a positive value of RACI.

4. CONCLUSION

In conclusion, the results revealed a significant influence of Spanish style processing on the phenolic compounds and antioxidant capacity of green table olives extracts of seven Algerian cultivars. This effect differs greatly among the cultivars; *Sigoise* is the most conservative variety and *Azzeradj* from Seddouk is the most dissipating one.

The results obtained for the antioxidant activity assessed by the four assays showed that among the studied cultivars, the fresh olives of the *Azzeradj* variety from Seddouk exhibited the highest phenolic content and exerted the strongest antioxidant activities, but they were also the most affected by the processing

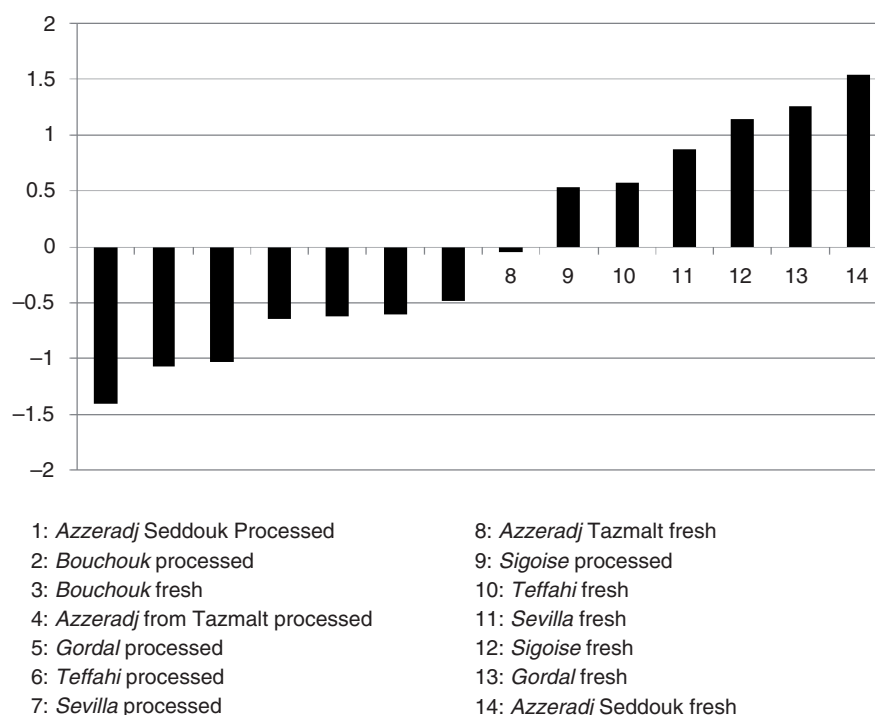


FIGURE 1. Relative antioxidant capacity index (RACI) of extracts of fresh and processed olives.

treatment (loss of 94.80% in phenolic content), which caused a loss of 93.74, 92.75, 97.94, 51.66% in reducing capacity, scavenging capacity against DPPH radical, hydrogen peroxide radical and in the bleaching test, respectively. Instead, *Sigoise*, the most representative cultivar of the Algerian Market, was the least affected by the processing. The antioxidant activity of processed olives recorded significant losses for the aqueous medium in contrast to the organic medium. Fresh olives of *Azzeradj* from Seddouk exhibited the highest RACI value, confirming their superiority in antioxidant capacity.

The data from this study show that olive extracts and mainly extracts of fresh olives of *Azzeradj* from seddouk may constitute a good source of healthy compounds. It would be interesting to use other methods of preparation that could preserve them.

As far as we know, this is the first report considering the antioxidant potential of Algerian green olive cultivars. Further studies are needed to focus on phenolic loss reduction as a result of Spanish style processing of green table olives.

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