



## Ortho-diphenol profile and antioxidant activity of Algerian black olive cultivars: Effect of dry salting process



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### ABSTRACT

Six Algerian olive cultivars (Azeradj, Sigoise, Bouchouk, Abelout, Aberkane and Atefah) processed by dry salting were investigated for the total polyphenols, *ortho*-diphenol profile and antioxidant activity. The dry salting affects total polyphenol and *o*-diphenol contents with a loss rate of 6–46% and 7–50%, respectively, depending on the cultivar. Consequently, a decrease in the antioxidant activity was observed, 10–35% for the reducing power, 29–58% for the DPPH radical scavenging activity and 10–48% for the ferrous-chelating power. Among the *o*-diphenols identified in the salted olives, hydroxytyrosol was the most abundant followed by verbascoside and caffeic acid. The comparative study showed that Sigoise from Relizane which contains the highest levels of polyphenols and *o*-diphenols exhibits the best antioxidant activity. Our results suggest that in addition to the processing, the cultivar and the geographical origin would have a pronounced influence on both *o*-diphenol composition and antioxidant activity of olives.

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### 1. Introduction

The olive tree (*Olea europaea*) is widely cultivated in many regions of the world where climatic conditions are as favourable as those prevailing in the Mediterranean countries. Algeria's olive crop area was around 188,923 ha by 2011. The total table olive production was estimated to 192,785 ton in 2011 (ITAFV, 2011). The olives grown in Algeria belong to a wide range of cultivars including Azeradj, Bouchouk, Aberkane and Atefah.

Numerous epidemiological surveys have shown an inverse relationship between the intake of fruits and the incidence of coronary heart disease and certain cancers. Many constituents of these dietary components such as polyphenols might contribute to their protective role (Rice-Evans, Miller, & Paganga, 1997). The consumption of table olives provides a large amount of natural antioxidants which play a major role in the antioxidant activity and in the prevention of many diseases (Boskou et al., 2006). Among olive polyphenols, *ortho*-diphenols such as hydroxytyrosol, oleuropein and verbascoside (Ryan & Robards, 1998) are recognized as the most important in relation to their antioxidant activity which can be related to hydrogen donation, i.e., their ability to improve radical stability by forming an intra-molecular hydrogen bond between the hydrogen of their hydroxyl group and their phenoxyl

radicals (Visioli & Galli, 1998). Several studies have demonstrated that phenolic content in table olives depends on the processing method (Ben Othman, Roblain, Chammen, Thonart, & Hamdi, 2009; Bianchi, 2003; Romero et al., 2004). Three types of table olives are of a great importance in the international trade and are mainly produced on an industrial scale: Spanish-style green olives, Greek-style naturally black olives and Californian-style black olives (Garrido-Fernández, Fernández-Díez, & Adams, 1997). However, there are some traditional preparations that have not attracted much attention. One of these involves the use of dry salt to eliminate the natural bitterness of the fruits and to make them edible.

The processing of the raw olives causes considerable modifications of the phenolic profile. This affects both the organoleptic properties and the antioxidant capacity of the finished product. Olive samples subjected to the same processing method react differently, depending on their varietal, chemical and physical characteristics (Bianchi, 2003). For this, it would be interesting to monitor the qualitative and quantitative evolution of phenolics of the table olives processed according to traditional methods. Consequently, the purpose of this study is to investigate the total polyphenols, *o*-diphenol composition changes after dry salting of six black olive cultivars and to determine their antioxidant capacity in order to evaluate the effect of such process on the *o*-diphenol profile and antioxidant activity of the finished product, because of the limited data on this kind of processing.

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## 2. Materials and methods

### 2.1. Olive samples

Six black olive cultivars (Azeradj, Sigoise, Bouchouk, Abelout, Aberkane and Atefah) harvested at the fully ripe stage were hand-picked from different parts of olive trees on December 2010. Sigoise samples were harvested from three locations (Mascara, Relizane and Oran) (Table 1).

### 2.2. Processing of olive samples

The collected olives (at least 2 kg) were treated with alternating layers of dry salt (0.8 kg), into baskets, and kept at room temperature for 30–50 days depending on the cultivar (Panagou, 2006). The salting caused dehydration and the olives appear shriveled. The obtained olive pulps were freeze-dried (Christ, Alpha 1–4 LDplus, Osterode am Harz, Germany), then ground in electric blender (IKA model A 11 B, Staufen, Germany) and stored at  $-18\text{ }^{\circ}\text{C}$  until analysis.

### 2.3. Extract preparation

Freeze dried table olive pulps (100 mg) were homogenized in 10 mL of 50% acetone. After stirring for 30 min, the mixture was centrifuged (nüve NF 200, Ankara, Turkey) at  $2800\times g$  for 20 min. The supernatant was collected and filtered, and the residue was re-extracted. The filtered extracts were combined and washed with hexane ( $5\times 10\text{ mL}$ ), then kept in refrigerator until analysis (McDonald, Prenzler, Antolovich, & Robards, 2001).

### 2.4. Analysis of phenolic compounds

#### 2.4.1. Total phenolic compounds

The amount of total phenolics in extracts was determined according to the method of Kahkönen et al. (1999). Aliquots (200  $\mu\text{L}$ ) of extract were mixed with 1.0 mL of Folin–Ciocalteu reagent and 800  $\mu\text{L}$  of sodium carbonate (7.5%). After incubation for 30 min, the absorbance was measured at 725 nm (Uvimini1240 spectrophotometer, Shimadzu, Suzhou, China). The total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100 g of dry weight.

#### 2.4.2. Total ortho-diphenol content

A mixture of 2 mL of the olive extract and 500  $\mu\text{L}$  of a 5% solution of sodium molybdate, was shaken vigorously. After incubation for 15 min, the absorbance was measured at 370 nm and the

results were expressed as mg of caffeic acid equivalents/100 g of dry weight (Bendini et al., 2003).

#### 2.4.3. HPLC analysis of ortho-diphenols

The preparation of extracts was based on the methodology proposed by Sánchez, Romero, Ramírez, and Brenes (2013). Freeze dried olive pulps (1 g) were homogenized with 6 mL of dimethylsulfoxide (DMSO). After stirring for 2 min, the mixture was centrifuged at  $28,000\times g$  for 6 min at  $22\text{ }^{\circ}\text{C}$ ; the supernatant was collected and filtered through a 0.22  $\mu\text{m}$  nylon filter. An aliquot of filtrate (250  $\mu\text{L}$ ) was homogenized with 250  $\mu\text{L}$  of internal standard (syringic acid 0.2 mM in DMSO) and 500  $\mu\text{L}$  of DMSO. A volume of this mixture (20  $\mu\text{L}$ ) was injected for HPLC analysis; a flow rate of 1 mL/min and a temperature of  $35\text{ }^{\circ}\text{C}$  were used.

The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600 E pump, a Waters column heater module, and a Waters 996 photodiode array detector operated with Empower software (Waters Inc). A 25 cm  $\times$  4.6 mm i.d., 5  $\mu\text{m}$ , Spherisorb ODS-2 (Waters Inc) column was used. The separation was achieved by gradient elution using an initial composition of 90% water (pH 2.5 adjusted with 0.15% phosphoric acid) and 10% methanol. The concentration of the latter solvent was increased to 30% in 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% in 10 min, which was maintained for 5 min. Finally, the methanol concentration for the last three steps was increased to 60, 70, and 100% in 5 min periods. Initial conditions were reached in 15 min. An injection volume of 20  $\mu\text{L}$ , a flow rate of 1 mL/min, and a temperature of  $35\text{ }^{\circ}\text{C}$  were used. Chromatograms were recorded at 280 nm (Romero, Brenes, García, & Garrido, 2002).

The concentration of each compound was calculated using a standard curve. Hydroxytyrosol, oleuropein and verbascoside were purchased from Extrasynthese SA (Lyon Nord, Genay, France). Hydroxytyrosol-1-glucoside and caffeoyl ester were quantified using the response factors of hydroxytyrosol and caffeic acid, respectively.

### 2.5. Antioxidant activity

#### 2.5.1. Reducing power

The reducing power was estimated using the procedure described by Gülçin, Oktay, Küfrevioğlu, and Aslan (2002). A volume of olive extract (250  $\mu\text{L}$ ) was mixed with 250  $\mu\text{L}$  of phosphate buffer (0.2 M, pH 6.6) and 250  $\mu\text{L}$  of potassium ferricyanide (1%). The mixture was incubated at  $50\text{ }^{\circ}\text{C}$  for 20 min. Aliquot (250  $\mu\text{L}$ ) of trichloroacetic acid (10%) and 200  $\mu\text{L}$  of ferric chloride (0.1%) were added to the mixture. The absorbance was measured at 700 nm

**Table 1**  
Total phenolic compounds and o-diphenols contents of studied olives.

Cultivar	Origin	Code	Total phenolic compounds <sup>1</sup>		Total o-diphenols <sup>2</sup>	
			Fresh olives	Salted olives	Fresh olives	Salted olives
Abelout	Béjaia	BT	2281.68 $\pm$ 14.62 <sup>cA</sup>	1283.28 $\pm$ 53.02 <sup>eB</sup>	1008.06 $\pm$ 09.49 <sup>eA</sup>	625.84 $\pm$ 28.13 <sup>fB</sup>
Aberkane	Béjaia	BK	2314.39 $\pm$ 68.45 <sup>cA</sup>	1548.48 $\pm$ 0.86 <sup>dB</sup>	1043.26 $\pm$ 03.02 <sup>eA</sup>	865.65 $\pm$ 19.80 <sup>eB</sup>
Azeradj	Béjaia	AZ	3782.02 $\pm$ 128.35 <sup>bA</sup>	2219.05 $\pm$ 102.32 <sup>bB</sup>	2115.39 $\pm$ 11.62 <sup>aA</sup>	1070.64 $\pm$ 07.72 <sup>bB</sup>
Bouchouk	Béjaia	B	1197.18 $\pm$ 66.24 <sup>eA</sup>	1028.82 $\pm$ 59.04 <sup>fA</sup>	845.67 $\pm$ 0.90 <sup>hA</sup>	585.57 $\pm$ 03.59 <sup>gB</sup>
Sigoise	Mascara	S1	3726.69 $\pm$ 73.43 <sup>bA</sup>	2007.26 $\pm$ 91.21 <sup>cB</sup>	1402.77 $\pm$ 1.31 <sup>cA</sup>	899.99 $\pm$ 20.83 <sup>dB</sup>
Sigoise	Relizane	S2	4355.02 $\pm$ 191.72 <sup>aA</sup>	2716.15 $\pm$ 11.63 <sup>aB</sup>	1601.77 $\pm$ 05.50 <sup>bA</sup>	1296.47 $\pm$ 03.27 <sup>aB</sup>
Sigoise	Oran	S3	3662.41 $\pm$ 58.17 <sup>bA</sup>	2154.29 $\pm$ 69.91 <sup>bB</sup>	1103.63 $\pm$ 0.74 <sup>dA</sup>	1022.12 $\pm$ 22.34 <sup>cB</sup>
Atefah	Béjaia	T	1684.11 $\pm$ 84.70 <sup>dA</sup>	1217.10 $\pm$ 74.58 <sup>eB</sup>	1026.50 $\pm$ 0.07 <sup>fA</sup>	511.27 $\pm$ 01.82 <sup>hB</sup>

<sup>A</sup> and <sup>B</sup>: within a row (effect of processing), different letters indicate statistically significant differences ( $p < 0.05$ ).

<sup>a–h</sup>: Within a column, different letters indicate statistically significant differences ( $p < 0.05$ ) between cultivars.

<sup>1</sup> Results in mg GAE/100 g dw are expressed as the average  $\pm$  Standard deviation of three replicates.

<sup>2</sup> Results in mg caffeic acid/100 g dw are expressed as the average  $\pm$  Standard deviation of three replicates.

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