



# Characterization of Phenolic Profile and Antioxidant Potential of Some Algerian Olive Oils Cultivars

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## HIGHLIGHTS

- Phenolic profiles and antioxidant activities of three Algerian olive oil cultivars were significantly different.
- Total phenolics of the studied cultivars were ranged from 132.8 to 318.9 mg gallic acid equivalents/kg.
- The ortho-diphenols contents varied between 13.02 and 31.1 mg caffeic acid equivalents/kg.
- Chemlal and Azeradj Algerian olive oil cultivars were considered as appropriate sources of bioactive phytochemicals.

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## Acronyms and abbreviations

DPPH=1,1-diphenyl-2-picrylhydrazyl  
GAE=Gallic Acid Equivalents  
CAE=Caffeic Acid Equivalents  
QE=Quercetin Equivalents  
AAE=Ascorbic Acid Equivalents  
HPLC=High Performance Liquid Chromatography

## ABSTRACT

**Background:** Olive oil is a source of antioxidants and poses positive effects on human health. The objective of this study was firstly to identify and quantify the phenolics of some Algerian olive oils; secondly, the antioxidant activity of the samples was assessed.

**Methods:** The virgin olive oils used in this study were derived from three Algerian cultivars, including Azeradj, Bouchouk, and Chemlal. After preparation of the samples, the phenolic, ortho-diphenol and flavonol contents in addition to phenolic profile were determined. Also, antioxidant capacity, reducing power, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity values were analyzed. Statistical analysis of the data was done with Statistica 5.5 Fr.

**Results:** The phenolic contents of analyzed olive oils showed significant differences ( $p < 0.05$ ) between three different cultivars. The total phenolics of the studied cultivars were ranged from 132.8 to 318.9 mg Gallic Acid Equivalents (GAE)/kg. The ortho-diphenols contents varied between 13.02 and 31.1 mg caffeic acid equivalents/kg. Also, the total flavonol content was ranged from 6.5 to 13.6 mg quercetin equivalents/kg. On the other hand, significant differences ( $p < 0.05$ ) were found between phenolic profiles and antioxidant activity of the three Algerian olive oil cultivars.

**Conclusion:** Both Chemlal and Azeradj Algerian olive oil cultivars can be considered as appropriate sources of bioactive phytochemicals, which play a major role in human health as free radical scavenger and can replace synthetic antioxidant in the food products.

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## Introduction

Olive oil is a source of antioxidants and poses positive effects on human health, in particular to exert some of the cardio protective properties (Carrióna et al., 2016). A wide range of phenolic compounds, belonging to many

classes, including phenolic acids, alcohols, flavonoids, secoiridoids, and lignans have been identified in virgin olive oils which play a major role in the antioxidant activity (Bayram et al., 2013; Servili et al., 2004; Visioli and Galli, 1998).

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Many researchers have found some chemical differences in virgin olive oils samples based on their cultivar and/or geographical origin, suggesting that the oil antioxidant content is not constant (Allalout et al., 2009; Tovar et al., 2001). Therefore, the objective of the current study was firstly to identify and quantify the phenolics of Algerian olive oils obtained from the three most representative olive cultivars cultivated in other location rather than previously reported; secondly, the antioxidant activity of the samples was evaluated.

## Materials and methods

### *Olive oil samples*

The virgin olive oils used in the present study were derived from three Algerian cultivars, including Azeradj, Bouchouk, and Chemlal. The olive fruits were manually harvested on December 2014, from an orchard located in Sidi-Aich, Bejaia, Algeria. The raw olive samples were firstly milled in the hammer crusher. After that the olive paste was kneaded for 30 min and then warm water was added. After vertical centrifugation, the olive oil was obtained; and stored at 4 °C in the dark until analysis.

### *Extract preparation*

The extract preparation was carried out according to the method described by Tsimidou et al. (1992) with some modifications. Briefly, 2 g oil was dissolved in 1 ml *n*-hexane. The solution was extracted two times with 2 ml methanol/water mixture (60/40, v/v). After agitation, the sample was centrifuged, then washed with *n*-hexane and finally, the methanolic solution was collected.

### *Phenolic compounds*

#### *-Total phenolic compound content*

Total phenolic content was determined according to the method of Kahkonen et al. (1999). Two-hundred µl of extracts were mixed with 1 ml Folin-Ciocalteu reagent, and 800 µl sodium carbonate (7.5%). After 30 min, the absorbance was measured at 725 nm. The total phenolic content was expressed as mg of Gallic Acid Equivalents (GAE)/kg of oil.

#### *-Total ortho-diphenol content*

A mixture of 2 ml of olive oil extract and 500 µl sodium molybdate solution (5%) was shaken. After 15 min, the absorbance was measured at 350 nm and the results were expressed as mg of Caffeic Acid Equivalents (CAE)/kg of oil (Bendini et al., 2003).

#### *-Total flavonol content*

A volume of 500 µl aluminium chloride (2%) and 750 µl sodium acetate (50 g/l) were added to 500 µl extract. Absorbance of the mixture was measured at 440 nm, and

the flavonol content was calculated as mg of Quercetin Equivalents (QE)/kg of oil (Kumaran and Karunakaran, 2007).

#### *-Phenolic profile*

Phenolic extracts of olive oil samples were evaluated by reverse phase High Performance Liquid Chromatography (HPLC) system following the procedure described previously (Romero et al., 2007). At first, 0.6 g olive oil sample was extracted by 3×0.6 ml *N,N*-dimethylformamide. The mixture was stirred for 1 min and centrifuged at 7500 rpm for 5 min; the three obtained extracts were combined and mixed with 0.5 ml syringic acid (0.2 mM), and then washed with *n*-hexane (2×2 ml). Then, nitrogen was bubbled into the three extract during 15 min to remove the residual *n*-hexane. The extract was finally filtered through a 0.22 µm nylon filter and then injected into the HPLC chromatograph.

The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600E pump, and a Waters column heater module (Waters Inc., Mildford, MA, USA). A Spherisorb ODS-2 (5 µm, 25 cm×4.6 mm. D.; Waters Inc., Mildford, MA., USA) column was used. Separation was carried out by an elution gradient with an initial composition of 90% water as well as 10% methanol. The content of the latter solvent was raised to 30% over 10 min, and was maintained up to 20 min. Then, the methanol content was increased to 40% over 10 min, maintained for 5 min, and after that raised up to 50%. The methanol content was finally increased up to 60, 70, and 100% in 5-min periods. A flow rate of 1 ml/min and a temperature of 35 °C were used in all experiments. A Jasco FP-920 fluorescence detector as well as a waters 996 diode array detector (Jasco, Tokyo, Japan) was connected in series mode.

### *Antioxidant activity*

#### *-Reducing power*

A volume of olive oil extract (250 µl) was mixed with 250 µl potassium ferricyanide (1%) as well as 250 µl phosphate buffer (0.2 M, pH 6.6). The mixture was then incubated at 50 °C for about 20 min. Aliquot (250 µl) of trichloroacetic acid (10%) and 200 µl ferric chloride (0.1%) were added to the mixture. The absorbance was measured at 700 nm; the results were calculated as mg of Ascorbic Acid Equivalents (AAE)/kg of oil (Gulcin et al., 2002).

#### *-Total antioxidant capacity (phosphomolybdenum assay)*

Total antioxidant capacity of olive oil extracts has been assessed according to Prieto et al. (1999); briefly, olive oil extract (100 µl) was mixed with 1 ml reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95 °C for 90 min and the absorbance was measured at 695 nm.

Total antioxidant activity was expressed as mg of AAE/kg of oil.

*-1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity*

One hundred  $\mu$ l olive oil extract was mixed with 900  $\mu$ l of 60  $\mu$ M DPPH solution. The reaction mixture was mixed by vortex and put in dark for about 30 min. The absorbance was evaluated at 517 nm. The antiradical activity was expressed as inhibition percentage (Lesage-Meessen et al., 2001).

#### Statistical analysis

Results were expressed as means $\pm$ standard deviation (SD). Statistical analysis of the data was carried out with Statistica 5.5 Fr. Analysis of Variance (ANOVA) was performed to estimate the statistically significant differences between olive oil samples for each parameter. *P* values <0.05 were regarded as significant levels in the experiments.

## Results

As indicated in Table 1, the phenolic contents of analyzed olive oils showed significant differences ( $p < 0.05$ ) between three different cultivars, including Azeradj, Bouchouk, and Chemlal. The total phenolics of the studied cultivars were ranged from 132.8 to 318.9 mg GAE/kg. The ortho-diphenols contents varied between 13.02 and 31.1 mg CAE/kg, while, the total flavonol content was ranged from 6.5 to 13.6 mg QE/kg. Significant differences ( $p < 0.05$ ) were found between phenolic profiles of the three olive oil cultivars (Table 2).

As shown in Table 3, significant differences in the antioxidant activity ( $p < 0.05$ ) were noted between studied oil cultivars. Azeradj extracts exhibited the best reducing power followed by Bouchouk and Chemlal. The results of the phosphomolybdenum assay were ranged between 72.1 and 96.7 mg AAE/kg. Chemlal cultivar extracts exhibited the best DPPH radical scavenging activity, followed by Azeradj and Bouchouk.

**Table 1:** Phenolic contents of three different Algerian olive oil cultivars

	Azeradj	Bouchouk	Chemlal
Total phenolics (mg GAE/kg)	318.9 $\pm$ 0.07 <sup>a</sup>	132.8 $\pm$ 0.9 <sup>c</sup>	221.3 $\pm$ 0.9 <sup>b</sup>
Total ortho-diphenol (mg CAE/kg)	31.1 $\pm$ 0.2 <sup>a</sup>	13.2 $\pm$ 0.2 <sup>b</sup>	30.7 $\pm$ 0.4 <sup>a</sup>
Total flavonols (mg QE/kg)	13.1 $\pm$ 0.1 <sup>a</sup>	6.5 $\pm$ 0.7 <sup>b</sup>	13.6 $\pm$ 0.2 <sup>a</sup>

Different letters within each row indicate statistically significant difference ( $p < 0.05$ )

**Table 2:** Phenolic profile of three different Algerian olive oil cultivars

	Azeradj	Bouchouk	Chemlal
hydroxytyrosol	4.03 $\pm$ 0.33 <sup>b</sup>	0.22 $\pm$ 0.01 <sup>c</sup>	5.91 $\pm$ 0.59 <sup>a</sup>
tyrosol	28.25 $\pm$ 4.37 <sup>a</sup>	3.12 $\pm$ 0.06 <sup>c</sup>	13.40 $\pm$ 0.08 <sup>b</sup>
pinoresinol	18.40 $\pm$ 2.34 <sup>b</sup>	11.62 $\pm$ 0.17 <sup>c</sup>	67.69 $\pm$ 1.00 <sup>a</sup>
1- Acetoxypinoresinol	42.04 $\pm$ 6.01 <sup>a</sup>	6.42 $\pm$ 0.18 <sup>c</sup>	27.75 $\pm$ 0.38 <sup>b</sup>
elenolic acid linked to hydroxytyrosol	17.06 $\pm$ 0.1 <sup>b</sup>	2.48 $\pm$ 0.23 <sup>c</sup>	37.65 $\pm$ 2.30 <sup>a</sup>
elenolic acid linked to tyrosol	48.54 $\pm$ 3.34 <sup>a</sup>	ND	34.27 $\pm$ 0.34 <sup>b</sup>
vanillic acid	0.53 $\pm$ 0.22 <sup>a</sup>	ND	0.61 $\pm$ 0.02 <sup>a</sup>
p-coumaric acid	0.72 $\pm$ 0.15 <sup>a</sup>	0.27 $\pm$ 0.04 <sup>b</sup>	0.34 $\pm$ 0.02 <sup>b</sup>
total phenols	159.57 $\pm$ 16.22 <sup>a</sup>	24.12 $\pm$ 0.59 <sup>b</sup>	187.62 $\pm$ 4.49 <sup>a</sup>

The values are shown as mg/kg

Different letters within each row indicate statistically significant difference ( $p < 0.05$ )

ND: Not Detected

**Table 3:** Antioxidant activity of three different Algerian olive oil cultivars

	Azeradj	Bouchouk	Chemlal
Reducing power (mg AAE/kg)	232.1 $\pm$ 0.8 <sup>a</sup>	116.6 $\pm$ 0.7 <sup>b</sup>	99.3 $\pm$ 0.2 <sup>c</sup>
Phosphomolybdenum assay (mg AAE/kg)	94.1 $\pm$ 0.6 <sup>a</sup>	96.7 $\pm$ 0.6 <sup>a</sup>	72.1 $\pm$ 0.9 <sup>b</sup>
Antiradical activity (%)	39.76 $\pm$ 0.16 <sup>b</sup>	22.13 $\pm$ 0.16 <sup>c</sup>	46.30 $\pm$ 0.12 <sup>a</sup>

Different letters within each row indicate statistically significant difference ( $p < 0.05$ )

## Discussion

In the current study, the total phenolic contents of Algerian olive oil cultivars are similar to those reported for some Spanish and Greek cultivars (Allalout et al., 2009). Also, the total ortho-diphenol contents acquired in this work are in agreement with those obtained by Bubola et al. (2014) for Croatian virgin olive oils. Indeed, Algerian cultivars analyzed in the current study contained similar amounts of total phenolics previously reported for cultivars from other Mediterranean countries (Servili et al., 2004; Visioli and Galli, 2002). However, the differences in phenolic contents of the olive oil cultivars of the present investigation are probably related to the cultivar, since all studied olive oils derived from olives which are cultivated under similar conditions, and the same extraction method is adopted to extract the oils.

The HPLC analysis revealed that tyrosol content (3.12 to 28.25 mg/kg) was too higher than those obtained for Turkish olive oil cultivars which were ranged from 0.18 to 1.57 mg/kg (Dağdelen et al., 2013). The notable tyrosol content of the studied cultivars could be useful for future selection of these Algerian olives to obtain oils which are more abundant in a particular simple phenol compound. On the other hand, we noted that the content of hydroxytyrosol was lower than of tyrosol; this observation is different from that of some Tunisian cultivars studied by Ouni et al. (2011). Concerning the phenolic acid, the levels are similar to those recorded by Nieves Franco et al. (2014) for Spanish cultivars. The present investigation showed higher contents of lignans than those of other investigators (Bayram et al., 2013; Nieves Franco et al., 2014) which is probably due to the samples origin.

The variations noted between the reducing powers of studied oils may be related to the phenolic contents because of the role of such compounds in reducing capacity. Also, the presence of other reducing agents (sugars, protein, etc.) can contribute to these differences, since it is characteristic to each cultivar. The quantitative and qualitative variations in phenolics between the investigated olive oil extracts could explain the observed changes in their antioxidant activities which are based on the transfer of electrons/hydrogen from antioxidants (Loo et al., 2008).

The results of phosphomolybdenum method carried out in this study indicated that Bouchouk cultivar which contains low levels of phenolics, showed a high total antioxidant capacity; this could be explained by its high content of reducing compounds rather than phenolic constituents. The variations in radical scavenging activity found between the tested extracts are probably related to the phenolic compound composition of the cultivars, particularly ortho-diphenols since such structure enhanc-

es their ability to act as efficient antioxidant (Tuck and Hayball, 2002; Visioli and Galli, 1998). The current investigation revealed that the olive oil derived from cultivar with a high content of hydroxytyrosol and its derivatives possess much greater antioxidant activity to scavenge the free radicals comparing to those with high content of tyrosol and its derivatives.

## Conclusion

This study provides new information about the phenolic profile and antioxidant capacity of olive oils derived from three most representative cultivars in Algeria. Qualitative and quantitative differences in phenolics were noted between the studied oils. Overall, the phenolics quantified, mainly belong to secoiridoid phenolics, phenolic alcohols, and lignans which represent the major constituents, mainly the pinoresinol. The comparative study indicated that both Chemlal and Azeradj cultivars can be considered as appropriate sources of bioactive phytochemicals, which play a major role in human health as free radical scavenger and can replace synthetic antioxidant in the food products; for this, it will be interesting to promote the culture of olives derived from these cultivars. In order to give more information about the authenticity and quality of the studied olive oils, the determination of aromatic profile should be performed to complete the present investigation.

## Author contributions

O.S. and H.L. designed the study and wrote the manuscript; C.R., M.H., K.H. conducted the experimental work. All authors revised and approved the final manuscript.

## Conflicts of interest

There are no conflicts of interest.

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