Investigation of antioxidant and antihemolytic activities of Algerian defatted olive fruits (*olea europaea* L.) at two ripening stages

Faiza Benlarbi\(^a,b,\ast\), Pierre Stoker\(^c\) and Mohamed Youssi\(^a\)

\(^a\)Laboratoire des Sciences Fondamentales-Université Amar Telidji, BP 37 G Laghouat, Algérie
\(^b\)Laboratoire de Valorisation et Promotion des Ressources Sahariennes (LVPRS), université Khasdi Merbah, BP 511, Ouargla, Algérie
\(^c\)Aix Marseille Université, CNRS, Institut de Chimie Radicalaire, UMR 7273, Laboratoire Sondes Moléculaires en Biologie et Stress Oxydant, Service 522, Centre Scientifique de Saint-Jérôme, F-13397, Marseille Cedex 20, France

Received 24 October 2017
Accepted 1 May 2018

Abstract

**BACKGROUND:** Olive fruits have become extremely valuable because they have important nutrient properties and have shown positive benefits for human health. The chemical composition and organoleptic characteristics may be influenced by genotype and some agronomic factors like olive drupe harvesting date.

**OBJECTIVE:** In this study, the greatest interest is to clarify and to give more information for Algerian *Olea europaea* L. by investigation of their total phenolic and flavonoid contents, phenolic compounds identification, total antioxidant capacity and antihemolytic activity at two levels of the olive ripening process.

**METHODS:** Colorimetric methods were used to quantify total phenolic and flavonoid contents. The phenolic composition was done by HPLC technique. The antioxidant activity was assessed by measuring radical scavenging activity against 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) radical cation (ABTS\(^{\cdot+}\)) expressed as Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP) and antihemolytic activity.

**RESULTS:** The harvesting time effect on HPLC analysis of olive fruits was significant. Interestingly, the phenolic composition of the olive fruits differed greatly between samples. Two compounds usually not described in olive fruit were identified, namely morine dihydrate and coumarin. The amounts of total phenolics varied widely in the investigated extracts and ranged from 495 to 2325 mg GAE/100 g for black olives and from 865 to 2387 mg GAE/100 g for green olives, whereas the flavonoids content expressed as rutin equivalent per 100 gram of defatted dry matter was ranged between 155 and 354 mg/100 g for green olives and between 214 and 260 mg/100 g for black olives. The antioxidant activity of black and green olives ranged from 5.86 and 4.88 to 59.44 and 50.50 mM Trolox equivalents respectively, while ferric reducing antioxidant power (FRAP) was within the range of 0.36 and 0.31 to 4.41 and 3.04 mM Fe(II) respectively. Majority of extracts exhibited a beneficial antihemolytic effect.

**CONCLUSIONS:** Results showed that the level of maturation influences the chemical composition, antioxidant and bioactive properties. Consequently, this allows us to estimate the best and optimal harvest time.

Keywords: Defatted olive fruit, phenolic compounds, antioxidant activity, antihemolytic

\(*\)Corresponding author: Faiza Benlarbi, Laboratoire des Sciences Fondamentales, Université Amar Telidji, BP 37 G Laghouat, Algérie. Tel.: +213 663770442; E-mail: fbenlarbi@yahoo.fr.

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1. Introduction

Numerous epidemiological studies have shown that the Mediterranean Diet (MD) has become universally associated with its positive effects on longevity and reducing of chronic degenerative diseases and those related with aging [1]. The health-promoting properties of an MD rich in fruits and vegetables are attributed to the complex combination between phytonutrients contained in the dietary components. Table olives and olive oil are most important components of MD that offer exceptional and powerful health benefits [2].

The only one among the Oleacea species with edible fruit is the olive tree, *Olea europea* L. Cultivation began more than 6000 years ago in the Mediterranean regions, developed in Andalucia by Arabs and was then introduced to America, Asia, South Africa and Australia [3, 4]. Among the 1500 olive cultivars classified in the world, only approximately 100 are catalogued as most important cultivar producing varieties according to the usage of their fruits: table olive processing, oil extraction and dual use cultivars [3].

The olive is a drupe, oval in shape, and consists of the epicarp, or skin, mesocarp, or pulp, and the endocarp, or stone (pit) which contains the seed. The pulp is 66–85%, the pit (including the seed) is 13–30% and the skin is 1.5–3.5% of the fruit weight [5]. Three phases are frequently noted in the growth of the olive fruit: a development phase, during which increase of oleuropein occurs; a green maturation phase, corresponding to reduction in the levels of chlorophyll and oleuropein; and a black maturation phase, marked by the occurrence of anthocyanins and during which the oleuropein quantity continue to decrease [6].

The antioxidant property of phenolic compounds found in olive fruits can be responsible for the beneficial effects of the MD. In addition, the high content of monounsaturated fatty acids also has been reported to have beneficial effect in comparison with other fatty acids [7]. The phenolic composition of olives is very complex and can be varied according to the quantity and quality of phenolics. Qualitative and quantitative differences of phenolic contents in olive fruits have a direct influence on the chemical composition of olive products since it is dependent on the cultivar, the climatic conditions, the agronomic techniques, and the fruit ripening, which determine the nutritional quality, the functional and technological potential of the olive fruit [3, 8–10].

Phenolic compounds act as natural antioxidants with protect effect on products of olive trees from oxidation caused by oxygen of atmosphere [11]. In addition, they also play an important role in human nutrition as protective agents against numerous diseases, protecting the body tissues against oxidative alterations due to free radicals and other reactive species. Polyphenols intake is beneficial for human health because their antioxidant activity has been related to lower risk of cardiovascular diseases [12], protective effects against some types of cancer [13, 14] and inflammatory diseases [15].

The olive tree (*Olea europaea*) is particularly cultivated for oil and table olives production, which have an important economic value. According to the statistical data [16], the worldwide production of table olives is estimated at more than 2.55 million tons (2014–2015 seasons). The Algerian production is on the rise, from 207 360 tons (2013–2014) to 216 000 tons (2014–2015). In 2015-2016 season, Algeria contributes with 2.1% (83 500 tons) to world production of olive oil [17]. It is one of the countries where the table olive and olive oil productions were particularly increased in the last ten years. A national program of agricultural development was implemented for the creation of a million hectare, to start again Algerian oleiculture. This objective could be reached especially with the entry in production of new plantations, in particular those of the steppe and South areas of Algeria.

Generally, the phenolic compounds of *Olea europaea* L. products, such as olive oil and olives have been widely investigated for many years [18–25]. However, the Algerian olive fruit has not been studied to equal extent. There are only a few recent studies that present the total phenolic content and antioxidant capacity of the phenolic fraction of Algerian olive fruit varieties [26–28].

As we say, olive oil contains 2% of the total phenolic content of entire olives, while the residual 98% is present in olive mill waste [29]. For this reason, suitable interests should be required for the important bioactive compounds such as phenolics from the olive residues for potential uses in functional foods. Our choice for the present study is based on the similitude between defatted pulp of olive fruits and olive mill waste (solid waste) considered as
one of by-products of the olive industry, obtained after extraction of olive oil. The present study was conducted to investigate phenolic composition and antioxidant potential of four defatted olive pulp from Laghouat and Mascara (towns in Algeria). The phenolic profile was analyzed using high performance liquid chromatography-diode array detection (HPLC–DAD). Additionally, antioxidant capacities were estimated by using two widely known chemical methods (ABTS and FRAP) and biological assay based on whole blood resistance to free radical aggression (antihemolytic assay). The influence of the variety and the stage of maturation of olives on the phenolic compounds and antioxidant activity have been studied also. In addition, a possible correlation between phenolic composition and the antioxidant activity at different developmental stages has been investigated.

2. Materials and methods

2.1. Chemical reagents

All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland) and Merck (Germany).

2.2. Plant material and sample preparation

Green and black olive fruits were collected from four cultivars: three cultivars (dahbia, X1 and X2 cultivars) from Laghouat, department in southern Algeria located at 425 km south of Algiers (the capital of Algeria) and another cultivar (Sigoise) from Sig (Mascara Province), town in north-western of Algiers located at 323 km. Laghouat (latitude 33° 47′ (N); longitude 02° 52′ (E); altitude 750 m) is characterized by a cold desert climate (arid zone) and Sig (latitude 35° 31.70′ (N); longitude 0° 11.62′ (W); altitude 62 m) is characterized by a dry and hot semi-arid climate.

Before any treatment, the olive samples named L1, L2, L3 and S (see Table 1) were cleaned and dried at room temperature during few days. Then, they were stoned and pulp was dried with the drying oven at 60°C. The pulps were milled into powder using a hammer mill and extracted twice with n-heptane [30] by agitation at room temperature for 24 hours to remove unwanted fat. The defatted pulps were dried and used for total quantitative and qualitative analysis of phenolics compounds.

2.3. Extraction of phenolic compounds

The preparation of phenolic extracts was effectuated according to the procedure described by Amiot et al. [31] with some modifications. Two grams of defatted pulps were extracted for 48 hours with 100 ml of 80% (v/v) aqueous ethanol at room temperature. Another successive extraction with 50 ml of the same hydro alcoholic solution was carried out at room temperature for 24 hours. After elimination of ethanol under reduced pressure in a rotary evaporator at 40°C, four successive petroleum ether extractions allowed removal of residual pigments and lipids. Then, the aqueous solution was extracted for three times with ethyl acetate in presence of aqueous solution with 20% ammonium sulphate, and 2% of ortho-phosphoric acid solution. The ethyl acetate fraction is dried by addition of a sufficient amount of anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The extract was dried, dissolved in 10 ml of methanol and kept at –10°C.

2.4. Total phenolic compounds

Total phenolic contents of all extracts were determined using Folin–Ciocalteu reagent, following Singleton and Rossi method [32] with minor modifications. Aliquots (100 μL) of extract were mixed with 500 μL of diluted Folin–Ciocalteu reagent (1 : 10) and 2 mL of sodium carbonate (20%). After incubation for 120 min,
the absorbance was measured at 760 nm using a Milton Roy Spectronic 601 UV–Vis spectrophotometer (Milton Roy Company, USA). The total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100 g of defatted sample. All the measurements were carried out in triplicate.

2.5. Total flavonoid content

Total flavonoids were estimated using AlCl₃ complexation method [33]. One mL of diluted sample was separately mixed with 1 mL of 2% aluminium chloride methanolic solution. The absorbance was measured 15 min later at 430 nm with a Milton Roy Spectronic 601 UV–Vis spectrophotometer (Milton Roy Company, USA). A standard curve was generated using rutin and the Total Flavonoid Content (TFC) was expressed as mg Rutin Equivalents (RE) per 100 g of defatted sample. All the measurements were carried out in triplicate.

2.6. HPLC/DAD analysis of phenolic compounds

HPLC/DAD analyses were performed using a Hewlett–Packard HPLC System (Agilent 1100 series, Waldbronn, Germany) equipped with a quaternary pump (G1311A) with on-line degasser, auto-sampler, column oven and Diode Array UV/VIS Detector (DAD), scanning from 200 to 800 nm and the wavelength was then selected and fixed at 210 and 280 nm since many peaks could only be found under this condition. A non-polar reversed phase C18 column (250.0 mm × 4.6 mm, particle size 5 μm) was used for HPLC analysis. Elution was carried out at room temperature and utilized 0.05% (v/v) phosphoric acid in water as solvent A and acetonitrile (100%) as solvent B. The elution gradient program is as follows: 5.0% B (0–5 min), 5.0% B to 33.0% B (5–30 min), 33.0% B to 100.0% B (30–40 min), 100.0% B (40–45 min), at a flow rate of 1 mL/min. The injection volume was 20 μL. Identification of phenolic compounds was made by comparison of the retention times with those of standard compounds and by recording the UV spectra of the peaks areas from the chromatograms. The quantification of the individual phenolic compounds was realized by using the external calibration method. Data were expressed as mg of phenols per 100 g of defatted sample.

2.7. ABTS radical cation scavenging activity

The ABTS assay was used as described by Re et al. [34] with slight modifications. ABTS radical cation (ABTS⁺) was generated by the reaction of a 2 mM ABTS (2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) solution with 70 mM of potassium persulphate. The mixture was stored in the dark at room temperature for 24 h before use. Phosphate Buffered Saline (PBS) solution at pH 7.4 was used to dilute the ABTS⁺ solution to an initial absorbance of 0.700 ± 0.020 at 734 nm.

After addition of 10 μL of extract or Trolox standard to 2 mL of diluted ABTS⁺ solution, absorbance at λ = 734 nm was measured 5 min after initial mixing. The decrease in the optical density was used for calculating inhibition percentage at different concentrations. The applied formula is the following:

\[
\text{Inhibition \%} = 100 \times \frac{(OD_0 - OD)}{OD_0}
\]

where OD is the optical density (OD₀ at the initial time and OD at the final time). Inhibition % was plotted as a function of concentration of extracts and Trolox for standard reference data. Antioxidant capacity was calculated in terms of TEAC (Trolox equivalent antioxidant capacity) expressed as mM Trolox equivalents (mM TE). Measurements were carried out in triplicate.
2.8. Ferric reducing antioxidant power (FRAP)

The FRAP method measures the absorption change that appears when the TPTZ (2, 4, 6-tripyridyl-s-triazine)-Fe$^{3+}$ complex is reduced to the TPTZ-Fe$^{2+}$ form in the presence of antioxidant compounds [35]. Briefly, the FRAP reagent was freshly prepared by mixing 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution (10 mM TPTZ in 40 mM/HCl), 10 mL FeCl$_3$·6H$_2$O (20 mM) in a ratio of 10:1:1. Samples were dissolved in methanol. An aliquot of 10 μL of solution was mixed with 2 mL of FRAP reagent and the absorption of the reaction mixture was measured at 593 nm after 10 min. The calibration curve was plotted using FeSO$_4$·7H$_2$O solution, and the results were presented as mM Fe (II). Measurements were carried out in triplicate.

2.9. Antihemolytic activity

*In vitro* oxidative hemolysis of human blood was used to study the influence of free radicals on biological membranes and the defensive effect of phenolic compounds and known antioxidants. This assay investigated the total blood protecting effect against free radicals aggression in presence of phenolic extracts, adapting the procedure described by Djeridane et al. [36]. Sampling of human blood was carried out at the central blood center in Marseille (France) from healthy donors. The blood samples were diluted to 1/50 in phosphate buffer saline solution (pH = 7.4). Blood solutions (100 μL) were incubated with 85 μL of various amounts of phenolic extracts at 37°C for 15 min before being reacted with free radicals generated from the thermal decomposition of a 50 mM solution of 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH). The blood solution was pursued turbidimetrically by measuring the decrease in absorbance at 450 nm using a 96-well microplate reader. Results were expressed, for the time corresponding to 50% of maximal hemolysis (half-hemolysis time, HT$_{50}$ in min), which refers to whole blood resistance to free radical attack. A good resistance of whole blood corresponds to a high half-time of hemolysis and this last, will increase by adding an antiradical substance. Ascorbic acid was used as standard.

2.10. Statistical analysis

Experimental data was expressed as mean ± standard deviation (SD) of three independent experiments. Data were subjected to statistical analysis using the SPSS 24.0 statistical software for Windows (SPSS Inc, Chicago, IL, USA). The one-way analysis of variance (ANOVA) followed by Duncan multiple range test ($p<0.05$) were employed to study the differences between individual means. Simple associations between variables were calculated as the Pearson correlation.

3. Results and discussion

3.1. Total phenolic content

Total phenolic content present in extracts of defatted pulps is summarized in Table 1. The results showed that there are significant statistical differences between the investigated cultivars ($p<0.05$). The amounts of total phenolics varied widely in the investigated extracts and ranged from 865 (L3) to 2387 mg GAE/100 g (L1) for green olives and from 495 (S) to 2325 mg GAE/100 g (L1) for black olives. Independently of the stage of ripeness, results clearly showed that L1 had the highest total phenolic content. Total phenols content of the samples in this study are similar to those obtained previously [26, 37, 38] for some Italian, Turkish and Algerian cultivars, but higher than those recorded in previous studies on Italian, Tunisian and Turkish olive cultivars [39–41].

A comparison between the total content of phenolics in green and black olives shows that the amount of phenolic compounds is higher in green olives. We noted that according to each cultivar, ripening caused a variable and
### Table 1
Total phenol and flavonoid contents of studied defatted pulp of olive fruits harvested in two different stages

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Origin</th>
<th>Code</th>
<th>Green olives (mg GAE/100 g DW)</th>
<th>Black olives (mg GAE/100 g DW)</th>
<th>Total flavonoids (mg RE/100 g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total phenols</td>
<td>Total flavonoids</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Green olives</td>
<td>Black olives</td>
<td></td>
</tr>
<tr>
<td>Dahbia</td>
<td>Laghouat</td>
<td>L1</td>
<td>2387 ± 44^dA</td>
<td>2325 ± 33^dA</td>
<td>350 ± 9^aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X1</td>
<td>Laghouat</td>
<td>L2</td>
<td>1262 ± 68^dA</td>
<td>696 ± 78^bB</td>
<td>346 ± 9^aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X2</td>
<td>Laghouat</td>
<td>L3</td>
<td>865 ± 28^dA</td>
<td>628 ± 68^bB</td>
<td>155 ± 5^gB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sigoise</td>
<td>Mascara</td>
<td>S</td>
<td>1132 ± 28^dA</td>
<td>495 ± 55^dA</td>
<td>354 ± 5^aA</td>
</tr>
</tbody>
</table>

Data represent mean values for each sample ± standard deviation (n = 3). a–d: Within a column, different letters indicate statistically significant differences (one-way ANOVA and Duncan test, P < 0.05) between cultivars. A and B: within a row (ripening effect), different letters indicate statistically significant differences (one-way ANOVA and Duncan test, P < 0.05). DW dry weight of defatted sample.

significant decrease (p < 0.05) in the total phenolic content of L2, L3, and S cultivars (Table 1). In the case of the L1 cultivar, the total phenolic content decreased along the fruit maturation, although this diminution was not statistically significant. These obtained results are opposite of the results investigated by Boskou et al. and Owen et al. [21, 30] and similar to those of other authors [42–44], where, a decrease in phenolic contents has been reported with ripening of olive fruits. According to results available in literature for diverse cultivars [10, 44–46], this can be related to the individual phenolic compounds content, particularly to the pattern observed for oleuropein (the main phenolic in the first stages of maturation) drastically decreased during ripening. The oxidative and enzymatic deterioration of the phenols in the olive fruit increases with the harvest phase probably as a result of the advanced age of the fruit tissue [47]. Note that the total phenolic content of olive pulp varied also according to the conditions of extraction, processing method [48], agronomic parameters (cultivar, climate, agronomic techniques . . . ), maturation, geographical origin and fruit ripening [10, 31, 42, 46, 48].

### 3.2. Total flavonoid content

Several studies have indicated that flavonoids exhibit biological activities, with antiviral, antiallergenic, anti-inflammatory, and vasodilatation actions. However, greater interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to scavenging and reducing free radicals [49]. In this study, the TFC for each sample was calculated and presented in Table 1. There are significant differences among some investigated olive cultivars (p < 0.05). The difference in TFC of studied extracts varied from 155 mg RE/100 g (L3) to 354 mg RE/100 g (L1) in green olives. It is noticed also that TFC in the various extracts of L1, L2 and S cultivars was not statistically significant. Concerning total flavonoids present in black fruits, a clear, statistically significant difference between some cultivars was observed (Table 1). Therefore, these levels varied from 214 to 260 mg RE/100 g. The highest level of TFC was recorded in L1 followed by L2, L3, and the least was found in S extract.

According to the effect of maturation level, our results indicated that there is a significant decrease (p < 0.05) in TFC during fruit ripening for all cultivars, except for L3 extract, which TFC increase significantly.

The obtained results showed that more than 12 % and less than 40 % of the extracted phenolic substances in the extracts were of flavonoid origin. Consequently, it is concluded that the richness of the studied extracts by phenolics is not only due to the flavonoids, but it is also resulted from the presence of other families of polyphenols. By comparison, the obtained results are similar to those of Brahmi et al. [50] for some Tunisian cultivars (chemlali and Neb Jmel), but higher than those of Chetoui cultivar growing in Tunisia [40].

Flavonoid composition may represent a practical contribution to biochemical characterization of olive fruit varieties. In fact, flavonoids are present from the first stage (green olive) of the fruit formation throughout the two phases characterizing olive fruit maturation [51]. However, the diminution of the total phenolic and flavonoid contents in the fruits collected at second stage (black olive) is probably correlated with the increase...
of the hydrolytic enzymes activity during the ripening of fruits. Therefore, the participation of polyphenols and flavonoids in essential biological activities could maintain the hypothesis of defensive needs of the plant rising during fruit ripening [50].

3.3. Identification and quantification of phenolic compounds

The HPLC/DAD analysis of the studied samples revealed different chemical profiles. In fact, 13 phenolic compounds were identified and quantified (Table 2). The four analyzed varieties have shown the presence of phenolic compounds classes such as hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, coumarins and phenolic alcohols.

Oleuropein, one of the most important constituents of olive phenolic fraction, was identified in the extracts from defatted olive pulp. Oleuropein is able to confer resistance to disease and to insect infestation of the tree; furthermore, it is responsible for the bitter taste of olives and shows antioxidant properties due to its catechol moiety [45]. The analysis of Table 2 reveals significant quantitative differences in oleuropein contents among the different cultivars at two ripening stages. In fact, the amount of oleuropein ranged from 32.63 (S) to 1973.42 mg/100 g (L1) for green olives and from 75.50 (S) to 373.01 mg/100 g (L1) for black olives. It is noticed that oleuropein is most concentrated in the green defatted pulp of Dahbia variety (L1) growing in Laghouat. Several studies [8, 31, 42, 44, 46] reported that the oleuropein content in the olive fruits of different varieties decreased during the course of ripening which is in agreement with the obtained values for L1 and L3 cultivars. The change in oleuropein content may be related to the increased activity of hydrolytic enzymes during the black maturation, including enzymatic bioconversion to diverse derivatives and appearance of anthocyanins [42]. In the case of L2 and S varieties, the levels of oleuropein increased significantly during the fruit maturation. This may be attributed to a growth phase needs. Indeed, the irregular distribution of phenolic compounds in olive fruits is correlated with their different biosynthetic pathways that are affected by biological cycle of olive. However, the ripening stage, characteristics of cultivars, temperature, precipitation and their interactions are mainly responsible for the quantitative and qualitative variations observed during ripening of olive fruit [50]. Oleuropein possesses many beneficial pharmacological effects including cardioprotective effect, anti-inflammatory, inhibition of platelet aggregation, anti-atherogenic activity, anti-cancer, antimicrobial properties and neuroprotective effect [52].

Among the identified compounds, hydroxybenzoic acids were detected in olive fruits. Chlorogenic, syringic and vanillic acids were identified in the studied samples at different concentrations. Chlorogenic acid (5-caffeoylquinic acid), first identified in Olea europaea leaves, was revealed in olives for the first time by Ryan et al. [53] and is now confirmed and quantified in all analyzed samples, although in amounts below 10.82 mg/100 g. The results of this study confirm that the chlorogenic acid content in different extracts is higher than those available in literature for diverse cultivars [10, 50]. Concerning the effect of maturation level, the content of chlorogenic acid decreases significantly during fruit maturation of L1, L2 and L3 cultivars as also observed by Sousa et al. [44]. On the other hand, equal values were found in S extracts at two ripening stages. Furthermore, Cioffi et al. [39] reported that the syringic acid was not detected in olive pomace. However, in this study, it has been detected only in L1 sample (34.05 mg/100 g) at ripe phase. This value is higher than those found in previous study [54]. In addition, vanillic acid was not detected only in L1 sample at unripe stage. The amounts of vanillic acid varied significantly from 38.77 to 376.29 mg/100 g in black olive fruits and from 27.55 to 211.01 mg/100 g in green olive fruits. It is clear that the highest vanillic acid content was found in Sigoise variety (S), while the lowest in dhabia (L1). Moreover, the levels of vanillic acid increased along the fruit maturation, although this rise was statistically significant (Table 2). This is not in agreement with findings about Tunisian Chemlali olives reporting that vanillic acid practically remained constant and that their concentrations were lower than 5 mg/100 g during maturation [50, 55].

Hydroxycinnamic acids were identified in this study. They included ferulic, p-coumaric and sinapic acids. Ferulic and p-coumaric acids were detected in some cultivars at different concentrations ranged from 4.91 to 47.18 mg/100 g, according to the olive types, molecule and level of fruit maturation. Moreover, sinapic acid was
Table 2
Individual phenolic compounds contents (mg/100 g of dry weight of defatted sample) of defatted pulp of olive fruits harvested at two ripening stages

<table>
<thead>
<tr>
<th>Phenols</th>
<th>Green olives</th>
<th></th>
<th></th>
<th></th>
<th>Black olives</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
<td>L2</td>
<td>L3</td>
<td>S</td>
<td>L1</td>
<td>L2</td>
<td>L3</td>
<td>S</td>
</tr>
<tr>
<td>Tyrosol</td>
<td>10.95 ± 1.13d</td>
<td>nd</td>
<td>10.41 ± 0.80d</td>
<td>135.87 ± 2.50a</td>
<td>25.18 ± 0.75c</td>
<td>nd</td>
<td>9.58 ± 0.52d</td>
<td>31.97 ± 1.31b</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>8.32 ± 0.61b</td>
<td>6.61 ± 0.54c</td>
<td>10.82 ± 0.76g</td>
<td>8.20 ± 0.69b</td>
<td>4.09 ± 0.36d</td>
<td>7.79 ± 0.71b</td>
<td>6.63 ± 0.55c</td>
<td>8.20 ± 0.69b</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>4.88 ± 0.95b</td>
<td>nd</td>
<td>3.90 ± 0.36c</td>
<td>1.81 ± 0.27d</td>
<td>3.60 ± 0.53c</td>
<td>nd</td>
<td>10.76 ± 0.67a</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>nd</td>
<td>27.55 ± 1.03d</td>
<td>130.02 ± 2.66e</td>
<td>211.01 ± 1.72d</td>
<td>38.77 ± 0.68f</td>
<td>284.79 ± 4.31b</td>
<td>276.12 ± 5.36c</td>
<td>376.29 ± 2.58a</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>34.05 ± 0.84a</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>526.25 ± 24.95a</td>
<td>112.93 ± 1.67d</td>
<td>20.17 ± 1.04b</td>
<td>47.37 ± 1.11f</td>
<td>446.82 ± 15.48b</td>
<td>147.08 ± 2.60c</td>
<td>86.75 ± 1.56c</td>
<td>64.11 ± 1.02f</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>47.18 ± 3.69a</td>
<td>nd</td>
<td>nd</td>
<td>10.41 ± 0.52b</td>
<td>8.77 ± 0.68b</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>4.91 ± 0.81d</td>
<td>nd</td>
<td>13.47 ± 0.81a</td>
<td>nd</td>
<td>11.67 ± 0.58b</td>
<td>nd</td>
<td>6.11 ± 0.54c</td>
<td>nd</td>
</tr>
<tr>
<td>apigenin-7-O-glucoside</td>
<td>5.78 ± 1.13b</td>
<td>nd</td>
<td>nd</td>
<td>12.60 ± 0.60b</td>
<td>5.19 ± 0.27b</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Oleuropein</td>
<td>1973.42 ± 90.12a</td>
<td>142.85 ± 1.62c</td>
<td>473.74 ± 11.91b</td>
<td>32.63 ± 0.71f</td>
<td>373.01 ± 12.14c</td>
<td>235.87 ± 5.22d</td>
<td>208.80 ± 3.36d</td>
<td>75.50 ± 1.50d</td>
</tr>
<tr>
<td>Coumarin</td>
<td>20.58 ± 1.51a</td>
<td>1.31 ± 0.60c</td>
<td>2.81 ± 0.70b</td>
<td>0.76 ± 0.12c</td>
<td>1.48 ± 0.24c</td>
<td>0.72 ± 0.08c</td>
<td>nd</td>
<td>0.94 ± 0.06c</td>
</tr>
<tr>
<td>Morine dihydrat</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>4.75 ± 0.25a</td>
<td>nd</td>
<td>4.60 ± 0.53a</td>
<td>nd</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>nd</td>
<td>343.00 ± 38.97c</td>
<td>375.48 ± 13.79b</td>
<td>381.12 ± 5.30b</td>
<td>1571.50 ± 18.75a</td>
<td>nd</td>
<td>nd</td>
<td>161.88 ± 1.64d</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD (n = 3). Different letters within the same row indicate significant differences (one-way ANOVA and Duncan test, \( P < 0.05 \)). nd: not detected.
identified in all olive varieties but not at the two ripening periods for some. Best of our knowledge, this is the first study identifying sinapic acid in olive phenol fraction at high quantities ranged from 161.88 to 1571.50 mg/100 g for black olives and from 343 to 381.12 mg/100 g in unripe fruits. This phenolic acid was described in low amounts in some olive obtained from Turkish varieties [54]. According to our results, it is noted that during maturation the quantity of the sinapic acid of L2, L3 and S cultivars decreased significantly but for L1 cultivar, sinapic acid was obtained only at black stage.

In this study, four flavonoids were also identified and quantified. These detected flavonoids were divided in three groups: flavones (Apigenin 7-Oglucoside and morin dihydrate), flavonols (rutin) and flavanol (catechin). Rutin is the main flavonoid compound quantified in our study. The highest content of rutin was recorded in L1 cultivar (526.25 mg/100 g), while the lowest level was observed in L3 cultivar (20.17 mg/100 g) at green stage. A clear significant increase in the concentrations of this compound during fruit maturation has been observed for L2, L3 and S cultivars, while L1 cultivar demonstrated a significant decreasing. These obtained results are more important than those reported by Gómez-Rico et al. [46], who found lower values for rutin in some Spanish cultivars. Many biological effects have been attributed to this flavonol, which shows antioxidant, anti-inflammatory, anti-thrombotic, cytoprotective, vasoprotective and antimicrobial activities [45]. Other flavonoids were detected in some phenolic extracts at lower amount: Catechin, morine dihydrate and apigenin 7-Oglucoside. Catechin was detected in some samples, L2, S and L1 black fruits. The highest level of this flavonoid was detected in Sigoise (S) black fruit (10.76 mg/100 g) and the lowest was found in L1 (1.81 mg/100 g).

Let us now address the tyrosol which is a phenolic alcohol; usually present in olives and frequently detected in olive pulp. It has been widely studied, due to its important antioxidant activity and health-beneficial properties [45]. In the present study, it was detected in three cultivars (L1, L3 and S). The highest tyrosol content was in Sigoise (S) drupes while the lowest was in L3. Tyrosol contents of green olive cultivars ranged from 10.41 (L3) to 135.87 mg/100 g (S). However, in black varieties, tyrosol contents ranged from 9.58 to 31.97 mg/100 g. This is in accordance with previous studies on Turkish olive cultivars [38]. It is noted also that the tyrosol concentration decreased during fruit maturation of L3 and S samples but increased significantly during ripening of L1 cultivar. This increase may appear from hydrolysis of other compounds containing tyrosol or ligistroside transformation [55].

The presence of coumarin was confirmed in our investigated olive cultivars at lower amounts, significantly ranged from 0.72 mg/100 g to 20.58 mg/100 g. The content of this phenolic decreased significantly during the fruit ripening of L1 sample. This compound was identified and quantified as a minor component for the first time in Algerian olive fruits.

In general, the chemical structures and levels of phenolic compounds in olives are complex. The maturity stages of olive fruits of the four studied cultivars were characterized by significant quantitative and qualitative changes in phenolics compounds. These maybe related to biosynthetic pathways of phenolics, probably involved in defence mechanisms plant against microorganism attack. The obtained results clearly suggest also that the cultivar and the geographical origin of olive fruits would have a great influence on the phenol content, as was previously reported [10, 48, 55].

3.4. Antioxidant activities

Investigation of antioxidants in food has been developed by several methods. Since different antioxidant compounds can act through diverse mechanisms, single method can not entirely estimate the total antioxidant potential of foods [56]. Thus, to evaluate the antioxidant activity, selecting an appropriate assay is very important. In the present work, antiradical activity and reducing power were performed to evaluate this activity.
Antioxidant capacities of defatted pulp of olive fruits as measured using the ABTS and FRAP assays

<table>
<thead>
<tr>
<th></th>
<th>ABTS assay TEAC (mM)</th>
<th>FRAP assay (mM Fe(II))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Green olives</td>
<td>Black olives</td>
</tr>
<tr>
<td>L1</td>
<td>59.44 ± 0.87abA</td>
<td>50.50 ± 0.89abB</td>
</tr>
<tr>
<td>L2</td>
<td>16.36 ± 0.72abA</td>
<td>12.88 ± 0.79abB</td>
</tr>
<tr>
<td>L3</td>
<td>5.86 ± 0.96bcA</td>
<td>4.88 ± 0.63bcA</td>
</tr>
<tr>
<td>S</td>
<td>14.89 ± 0.95abA</td>
<td>9.80 ± 0.81bcB</td>
</tr>
<tr>
<td></td>
<td>Green olives</td>
<td>Black olives</td>
</tr>
<tr>
<td>L1</td>
<td>4.41 ± 0.52abA</td>
<td>3.04 ± 0.40abB</td>
</tr>
<tr>
<td>L2</td>
<td>0.93 ± 0.15abA</td>
<td>0.42 ± 0.07abB</td>
</tr>
<tr>
<td>L3</td>
<td>0.45 ± 0.09bcA</td>
<td>0.32 ± 0.06bcB</td>
</tr>
<tr>
<td>S</td>
<td>0.36 ± 0.05abA</td>
<td>0.31 ± 0.06abA</td>
</tr>
</tbody>
</table>

Data represent mean values for each sample ± standard deviation (n = 3). a–d: Within a column, different letters indicate statistically significant differences (one-way ANOVA and Duncan test, \( P < 0.05 \)) between cultivars. A and B: within a row (ripening effect), different letters indicate statistically significant differences (one-way ANOVA and Duncan test, \( P < 0.05 \)).

3.4.1. Radical scavenging activity against the radical ABTS\(^+\)

Antioxidant activities of the methanolic extracts from the selected samples were measured by applying the ABTS assay, expressed as TEAC (Trolox Equivalent Antioxidant Capacity) and summarized in Table 3. In fact the greatest value of the TEAC corresponds to the highest antioxidant activity. All samples were able to scavenge the ABTS\(^+\) radical cation. For the green fruits, the best ABTS\(^+\) scavenging activity was shown by the extract from L1 sample where TEAC value is 59.44 mM corresponding to a higher efficiency of the extract to neutralize the radical ABTS\(^+\). Whereas, the others extracts (L2, S and L3) showed a lower ABTS\(^+\) scavenging activity (16.36, 14.89 and 5.86 Mm respectively). Significant differences (\( P < 0.05 \)) were observed in the case of black olives where L1 extract has also the higher value of TEAC (50.5 Mm) followed by the other samples: L2 (12.88 mM), S (9.80 mM) and L3 (4.88 mM). The higher antioxidant capacity of L1 extract can be explained by the higher content of total phenols. The green types of all cultivars have a higher antioxidant activity than the black types. These findings suggest that phenolic compounds are the most important contributor to antioxidant capacity of olive samples investigated in this study. The obtained results are in agreement with those reported by Ben Othman et al. [43] and very important than those revealed by Pellegrini et al. [56].

3.4.2. Ferric reducing antioxidant power

In this study, antioxidant capacities of selected samples were also evaluated using the FRAP assay. This method was first developed to quantitate ascorbic acid in serum or plasma. In this assay, the antioxidant capacity is measured to estimate the ability of antioxidant molecules to reduce Fe\(^{3+}\)-TPTZ complex to the Fe\(^{2+}\) form under acidic conditions. By comparison to other tests of total antioxidant power, the FRAP assay is simple, rapid, inexpensive, very useful for routine laboratory analysis of antioxidant, and can be practiced to both aqueous and alcohol extracts of plants [35]. As seen from Table 3, the ferric reducing capacities of the studied samples ranged from 0.36 to 4.41 mM Fe\(^{2+}\) for green fruits and from 0.31 to 3.04 mM Fe\(^{2+}\) for black olives. These results reveal that both green and black olive extracts showed a marked capacity for iron reduction with values similar to those obtained by Ziogas et al. [57] for Amfissis cultivar of Greek origin. The reducing power of different examined extracts obtained from green olive fruits decreased significantly in the order of L1 > L2 > L3 > S. No significant differences were observed between L2 and L3 samples, also between L3 and S samples. Another classification was resulted for black olives, where the reducing capacities decreased in the following order: L1 > L2 > L3 > S. Significant differences (\( P < 0.05 \)) were noted between the L1 sample and others but it’s not the same case between L2, L3 and S samples (\( P > 0.05 \)). According to our results, it is clear that the extract L1 (green and black olives) exhibited the strongest efficiency (4.41 mM, 3.03 mM) and showed more powerful antioxidant than the other extracts. This result can be explained by the high phenolic content in this last cultivar at the two ripening stages as previously detailed. On the other hand, it is also concluded that the reducing capacities of L1, L2 and L3
samples decreases significantly \((p < 0.05)\) during the maturation of the fruit. This can be explained by some changes produced in the phenolic composition of fruit during maturation [58]. This could have a direct impact in reducing power.

3.5. Antihemolytic activity

Free radicals are highly reactive species produced under normal biologic conditions, essentially during oxygen consumption in redox reactions needed to produce energy, and remove xenobiotic and pathogenic organisms. An excess of free radicals can induce a pronounced impairment of the cellular metabolism and significant damage of tissues. Oxidative damage is increasingly recognized as responsible of numerous diseases such as cancer and cardiovascular diseases [59]. Total antiradical capacity of studied extracts was evaluated by a biologic test based on total blood resistance against free-radical attack. The Antihemolytic assay has already been used for a long time in evaluating free radical damages, and its inhibition by antioxidant has been recognized, it allows the measurement of the global antioxidant defence of animal and human blood against free radicals [60]. These can be generated by the thermal decomposition of AAPH (peroxyl radical initiator) and will attack erythrocytes, to stimulate the chain oxidation of protein and lipid, perturbing the organization of membrane and finally leading to hemolysis [61].

The defensive effect of the extract on total blood hemolysis by peroxyl radical scavenging activity was investigated in this study. The measurement of HT\(_{50}\) is very reproducible and has been shown to be representative of the overall defence against free radicals in humans and animal models. The activity of the extract was compared with that of ascorbic acid as antioxidant reference. The obtained results of lysis experiments at 10 and 100 \(\mu\)M are shown in Table 4 and expressed as a percentage of hemolysis inhibition \((\% HI)\), calculated with the following formula: \(\% HI = \frac{(HT_{50\text{sample}} - HT_{50\text{control}}) \times 100}{HT_{50\text{control}}}\). Where \(HT_{50\text{control}}\) is the half-hemolysis time of control (AAPH was used as hemolysis control) and \(HT_{50\text{sample}}\) is the half-hemolysis time in presence of sample. This inhibition percentage of hemolysis is an indication of the protection degree against the oxidative damage of cell membranes of whole blood (from healthy human donors), induced by AAPH. Different extracts exhibited a beneficial antihemolytic effect. For samples obtained from black olives, results indicated a very marked antioxidant effect of L1 (165.21%) and L2 (153.69%) extracts. This effect increases with the concentration and it is far stronger than that of antioxidant control (Ascorbic acid). It is remarkable that the L3 extract does not have an antioxidant activity. On the contrary, it becomes pro-oxidant starting from 10 \(\mu\)M and accelerates the hemolysis operation. This phenomenon (pro-oxidant activity) was observed also for S extract, but it starts from 100 \(\mu\)M. Moreover, all extracts obtained from green olives showed also an interesting anti-hemolysis efficiency against free radical damage, except the L3 extract, which becomes pro-oxidant at 100 \(\mu\)M. This can be explained by the effect that some phenolics may act as pro-oxidants under some circumstances such as at high concentration or in the presence of metal ions [62]. Another effect was noticed with the level of maturation. This effect indicated that the antihemolytic activity of different extracts at 10 \(\mu\)M is always highest for green olives, but at 100 \(\mu\)M this relationship is reversed. The high quantity of total phenolics in L1 extracts (green and black olives) suggests that the radicals can be destructed by the extracts before attacking the biomolecules of the blood cell membranes and causing oxidative hemolysis.

In this biological test, antioxidants such as polyphenol compounds extracted from olive fruits are supposed to inhibit free radical aggression causing lipid peroxidation and consequently red blood cells lysis. It is important to note that this test makes it possible to measure at the same time the antioxidant power of plasma and that of the components of red blood cells (RBCs) containing every type of the molecular and enzymatic antioxidants.

According to the results obtained by Paiva-Martins et al. [63], the interaction between phenolics and RBC proteins can inhibit hemolysis and oxidative damage of the RBC membrane provoked by peroxyl radicals using a different mechanism from the simple radical scavenging activity. One can assume that the ability to protect the RBCs from hemolysis is related not only to a radical scavenging activity but also to its ability to interact directly with cell membranes (in the outer or inner membrane surface), inducing modifications of the protein profile.
Table 4

Antihemolytic activity of phenolic extracts of defatted pulp of olive fruits

<table>
<thead>
<tr>
<th>Hemolysis inhibition (%)</th>
<th>Black olives</th>
<th>Green olives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 μM</td>
<td>100 μM</td>
</tr>
<tr>
<td>L1</td>
<td>51.65</td>
<td>165.21</td>
</tr>
<tr>
<td>L2</td>
<td>36.32</td>
<td>153.69</td>
</tr>
<tr>
<td>L3</td>
<td>−30.64</td>
<td>−67.51</td>
</tr>
<tr>
<td>S</td>
<td>25.68</td>
<td>−43.01</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>30</td>
<td>72</td>
</tr>
</tbody>
</table>

As previously reported by Tabart et al. [64], flavonols, anthocyanins, flavanons and phenolic acids are effective antioxidants protecting human RBCs against free radicals caused oxidative hemolysis and the defensive effect of flavonoids can be related to their binding to the plasma membrane and their facility to penetrate lipid bilayers.

The results of this test clearly indicate that some olive polyphenolic extracts exhibits strong antioxidant activity when tested in human whole blood, due to a cooperative antioxidant interaction between its polyphenol components. However, more in vivo studies are indispensable to explain the effect of olive polyphenols on indicators of oxidative stress, especially plasma isoprostane levels and DNA damage [65].

In general, the three employed methods clearly designated that the studied extracts have considerable antioxidant activities. Results differ depending on the test used. This can be explained by the sensitivity of each test to the analyzed extracts. The biological assay appears clinically more important than most other common assays because it can reveal numerous antioxidants classes [60].

3.6. Correlation analysis

In this section, the correlations between total phenolic and total flavonoid contents of Algerian olive fruit (defatted pulps) extracts and values obtained with the TEAC, FRAP and the antihemolytic assays have been investigated and some correlation coefficients have been calculated and reported in Tables 5 and 6.

3.6.1. Correlation of total phenolics and flavonoids with antioxidant assays

The results of Pearson correlation indicated linear positive relationships between the TEAC values and total phenolic contents, as well as between the FRAP values and the total phenolic contents, as shown in Table 5. TEAC values were highly correlated with total phenolic contents with significance levels for green olives ($r = 0.998, p < 0.01$) and black olives ($r = 0.986, p < 0.05$). In addition, on the basis of FRAP values. These were correlated positively with total phenolic content of green olives ($r = 0.982, p < 0.05$) and black olives ($r = 0.998, p < 0.01$).

The very strong relationships between the results obtained by the two methods (TEAC and FRAP) of measuring antioxidant activity and the total phenolic content, have shown that phenolic compounds largely contribute to the antioxidant activities of olive fruits, and therefore could play an important role in the beneficial effects of these fruits.

The obtained results of this study have indicated also that the flavonoid contents are in moderate correlations ($0.371 < r < 0.636$) with values of TEAC and FRAP but each one is not statically significant ($p > 0.05$). This means that the antioxidant activity is not only related to the flavonoid compounds but may be resulted from other phenolic compounds such as phenolic acids, tannic acid, proanthocyanidin, which also contribute to the antioxidant activity, depending on their chemical structures, number, and arrangement of the hydroxyl groups on the aromatic ring of the phenolic molecules.
The coefficients of correlation between the total phenolic content of the extracts and values obtained with the hemolysis assays were calculated at 10 μM of inhibitors (Table 5). The results have shown no significant correlations between antihemolytic activity and total phenolic content present in black and green olives. This is possibly due to the fact that plasma also takes part in the antioxidant power of whole blood by the presence of many antioxidants: water-soluble (ascorbic acid, uric acid, thiols and bilirubin), lipid-soluble (tocopherols, β-carotene and other carotenoids), proteins of plasma and antioxidants of membranes in which vitamin E plays a main role against oxidation [66]. The weak correlation could be related also to available coenzymatic activities which cannot be noted in chemical assays [53].

There are three reasons to explain the unclear relation between the antioxidant activity and total phenolics: (1) Antioxidants comprised not only total phenolics content; (2) the synergy between the phenolics in the mixture made the antioxidant capacity, not only reliant on the concentration of antioxidant, but also on the structure of phenolics [36]. That is why samples, L2 and L3 of green olives, with similar concentrations of total phenolics, may vary differently in their antioxidant activities (hemolysis and TEAC assays); (3) the antioxidant activity of phenolics is mostly due to their mechanism characteristics which make them act as hydrogen donors, reducing agents, singlet oxygen quenchers and also may have a potential to chelating metallic. Those various mechanisms may lead to different observations.

### 3.6.2. Correlation between the antioxidant assays

As exposed in Table 5, the antioxidant capacities obtained from the TEAC assay are in good accordance with those obtained from the FRAP assay, for green olives \( (r=0.985, p<0.05) \) and black olives...
(r = 0.991, p < 0.01). This implies that the antioxidants in these olive extracts are able to scavenge free radicals (ABTS⁺) and reduce oxidants (ferric ions), and confirms the results revealed by Prior et al. [67]. This is due to the similarity between the redox potentials (0.70 V for ferric reduction and 0.68 V for reaction with ABTS).

The results of the biological test and the two chemical tests, cannot certainly be directly compared, but bring interesting indications at the same time on the behaviour of the studied extracts and the knowledge of these methods. Therefore, it has been observed also that the antioxidant activities measured by TEAC, FRAP assays and those determined by hemolysis assay were not correlated significantly (p > 0.05) as shown in Table 5. The coefficients of correlation vary from 0.59 to 0.759. These values reveal that the biological test and the two chemical tests give different answers on the total antioxidant power of the studied extracts. The used chemical tests quantify a direct antioxidant effect (free radical scavenging activity and oxidant reducing power) whereas the bioassay brings an evaluation of the total antioxidant power. This will explain not only the direct effects of the various phenolic extracts, but also indirect effects (biological and enzymatic) strongly related to the presence of erythrocytes and plasma in diluted human blood.

3.6.3. Correlations between phenolics and antioxidant assays measured for green and black olive fruits

As shown in Table 6, the obtained results demonstrate a positive and statistically significant correlation (r = 0.968, P < 0.05) in terms of total phenolic contents between green and black olive fruits of all varieties. In addition, other strong and positive correlations (P < 0.01) were identified between total phenolic contents and antioxidant capacities measured by TEAC, FRAP and hemolysis assays. In this case, the correlation coefficients are 0.998, 0.996 and 0.998 respectively. These results enable us to say that information concerning the influence of maturation on the composition, antioxidant and bioactive properties of olives are most important which can permit us to better understand olives composition and consequently to estimate the best and optimal harvest time.

4. Conclusion

In conclusion, this study provided evidence that significant quantitative and qualitative changes in olive fruit phenolics are typically influenced by a range of factors, such as maturity level, cultivar, and climate. The results revealed a considerable influence of maturity level on the phenolic compounds and antioxidant capacity of four Algerian olive cultivars.

The study revealed that phenolic compounds in defatted pulp of olive fruit exhibit antioxidant and antihemolytic activities. Among the studied cultivars, the green olives of all varieties exhibited the highest phenolic content and exerted the strongest antioxidant activities, compared with black olives. Interestingly, the analyzed extracts revealed that the green type of Dahbia variety was found to have the highest polyphenolic contents and antioxidant activity compared to the fruits of other varieties. Therefore, one can say that some Algerian olive fruits represent an important source of antioxidant. The strongest correlation coefficients were established between the two assays (FRAP and ABTS) and phenolics.

As far as we know, this is the first chemical study of Dahbia variety growing in Laghouat (arid zone situated at 400 Km south of Algiers, Algeria). Findings suggest that defatted olive pulp of studied cultivars can be successfully used as natural, economical and promising source of phenolic compounds and antioxidant in pharmaceutical industry and can be used also as dietary supplements and food additives in nutraceutical and food industries. Further studies are needed to focus on Algerian olive cultivars, to confirm the obtained results and to better characterize the Algerian olive tree. More work therefore needs to be carried out on the other fractions to isolate, purify and characterize the active chemical compounds responsible for radical scavenging activity. Moreover, more studies are requested throughout many stages of maturation in order to totally understand the full impact of ripening in olive fruits composition.
Acknowledgments

The authors wish to thank the Algerian Ministry of High Education and Scientific Research for funding the Research Laboratory of Fundamental Sciences-Laghouat, Algeria.

References


