Effect of the harvesting period on the phenolic content and on antioxidant activity of two Algerian olive cultivars

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Because of their antioxidant and anti-free radical capacities, the phenolic compounds of olives gained growing interest. The aim of this work is to determine the harvest optimal period of two Algerian olive varieties *(Chemlal and Blanquette de Guelma)* harvested at four stages of maturity which will allow the best equilibrium between the olives' composition in phenolic compounds and their antioxidant activities.
Phenolic compounds were determined by colorimetric methods and by UPLC-PDA. The antioxidant activity of olive extracts was assessed by measuring the reducing power, the antiradical activity toward DPPH radical and ABTS.
Results confirm that the process of maturation and the variety of olives affect considerably the phenolic compounds and their antioxidant activity. As a consequence, a significant decrease in the antioxidant activity (reducing power and antiradical activities) of the olive fruits is observed. The relative antioxidant capacity index (RACI), which represents the mean scores of the antioxidant activities, showed that the highest indices were obtained by the green stage for *Chemlal* and spotted green stage for *Blanquette de Guelma*.
The harvesting can occur when the olives are in the spotted green stage, corresponding to the middle of December. This period allows a good combination between the phenolic content and antioxidant activity.

Keywords: Algerian olive cultivars, Harvesting time, Phenolic compounds, antioxidant activity

1. INTRODUCTION

The intense interest for the biological effects of natural antioxidants has been observed in recent years [1]. These antioxidants are included in quality of products and in the fight against oxidative stress (unbalanced production/removal of reactive oxygen species (ROS)) involved in the pathogenesis of several chronic diseases and ageing [2].
The olive tree (*Olea europaea*) have a significant economic importance for many countries of the Mediterranean Basin. Olives and olive-derived products are considered as functional foods; their properties are closely related to the quantity of monounsaturated fatty acids and minor compounds (vitamin E, polyphenols) [3]. Phenolic compounds are minor constituents of the olive fruit (comprising 1-3% of the fresh pulp weight) but have very important roles in their antioxidant, anti-inflammatory and anticarcinogenic activities [4]. They have also been associated with the prevention of cardiovascular and degenerative diseases [5].
Ripening is one of the most important factors associated with the quality evaluation of fruits and vegetables [6]. During maturation, Olive fruit undergoes a

series of changes including colour, firmness and the composition of the fruits. For oil-processed olives, the optimal harvest period was determined mainly by the maximum oil accumulation and the fruit resistance to detachment [7, 8]. However, maturation is one of the important factors affecting greatly the chemical composition of antioxidant compounds, particularly polyphenols [9]. The olives' maturation is accompanied by numerous hydrolysis reactions following the esterase and the β-glucosidase activation, which lead to the formation of simple phenols [10-12]. In general, during ripening, the amount of oleuropein and the concentration of most major phenol secoiridoids decreased [13-15] although the rate of change varied depending on cultivar and environmental conditions [16-17]. Simple phenolic compounds such as tyrosol and hydroxytyrosol that are considered as a degradation product of phenol secoiridoids displayed an irregular evolution depending on the variety and location [13-15, 18, 19].

Usually, late harvesting results in oils with the lowest hydrophilic phenols concentration [20]. In contrast, early harvesting, when the oil content is still increasing, results in oils with higher phenols content, which contributes to the level of bitterness and pungency [21]. It is important to determine the optimum maturity stage of olives for each individual cultivar to produce high-quality olive oil with an antioxidant profile leading to health benefits. The aim of this work is to determine the harvest optimal period of two olives varieties of the Bejaia region (*Blanquette de Guelma and Chemlal) at* four stages of maturity that will allow the best equilibrium between the olive composition in phenolic compounds and their antioxidant activities.

2. EXPERIMENTAL PART

The study was carried out during the 2013/2014 olive collection season. Olive fruit samples from two varieties (*Chemlal* and *Blanquette de Guelma*) were harvested by hand from all the floors of the foliage of five trees in the ITAFV (Technical Institute for Fruit Trees and Vine) located at Takarietz (Sidi-Aich, southern Béjaia), Algeria (36°, 36′, 47″ north and 4°, 41′, 18″ east, at an altitude of 111 m) at different dates corresponding to green, green-spotted, purple and black stages based on the degree of skin pigmentation. The dates of harvest are:

- *Chemlal* (C1: 01 December 2013; C2: 21 December 2013; C3: 12 January 2014; C4: 06 February 2014).
- *Blanquette de Guelma* (B1: 28 November 2013; B2: 16 December 2013; B3: 07 January 2014; B4: 29 January 2014).

The region of Bejaia has a temperate climate characterised by a mild winter typical of the Mediterranean areas with an average temperature of 15°C. The summer period has an average temperature of about 25°C. The annual rainfall amounts ranged between 600 to 1.100 mm in average. The plot received three gravity irrigation: late June-early July, late August and during the month of September.

After harvesting, the maturity index (MI) [22], Weight [23], moisture [24] and oil content [25] of the olives were determined.

Olive fruits were freeze-dried at –58°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°C until analysis.

EXTRACTION PROCEDURE: ULTRASOUND-ASSISTED EXTRACTION (USLE) - WITH ULTRASOUND BATH METHOD FROM OLIVE FRUIT SAMPLES

Extracts were prepared according to the method of Jerman *et al*. [26]. Extractions were carried out using 200 watts and 24 kHz UP200S ultrasonic system (Hielscher Ultrasonics GmbH, Teltow, Germany). Olive fruit freeze-dried powder (1.5 g) was sonicated in 25 mL of pure methanol (20 min, at 25°C). The extraction was repeated three times, during each the temperature of homogenate varied from 43 to 45°C.

The homogenates of each extraction step were centrifuged (Eppendorf, 9000 rpm, 5 min), supernatants decanted, merged and diluted with methanol to 100 mL. Prepared extracts were put in a screw-capped dark glass container and stored in the freezer (–25°C) until further UPLC analysis.

TOTAL PHENOLIC COMPOUNDS

The total phenolic compound contents of the extracts were determined [27]. After extraction, the total phenolic contents were determined spectrophotometrically at 765 nm using the Folin–Ciocalteu reagent and expressed as mg gallic acid equivalents (GAE) per 100 g of DM (olive samples) by referring to the calibration curve.

ORTHO-DIPHENOLIC COMPOUND

Ortho-diphenolic content was determined [28]. 0.5 mL of phenolic extract was dissolved in 5 mL of methanol-water (1:1, v/v); a mixture of 4 ml of the solution and 1 mL of 5% solution of sodium molybdate dihydrate in ethanol-water (1:1, v/v) was shaken vigorously. After 15 min, the absorbance at 370 nm was measured using caffeic acid for the calibration curve with a glass cuvette.

QUANTIFICATION OF INDIVIDUAL PHENOLIC COMPOUNDS BY UPLC-PDA

The determination of phenolic compounds was carried out on an ACQUITY UPLC® H-Class system coupled to an ACQUITY UPLC® Photodiode Array (PDA) detector and controlled by Empower™ 3 Chromatography Data Software (Waters Corporation, Milford, MA,

USA). The PDA was set in the wavelength range of 200-400 nm for the 3D scan with collection data rate at 40 pts s–1 to identify the compounds. Whereas, the PDA detector was set for the compound quantification at a fixed wavelength for 2D scan with a collection data rate at 80 pts s^{-1} at the maximum absorbance of the corresponding compounds and at 280, 300 and 320 nm for peak integrations. The identification of phenolics in the samples was achieved initially by comparison of retention times and maximum UV absorptions with those of standards then running spiking procedures with pure standards.

Separations of phenolics in 3.0 µL injected samples were performed at a temperature of 47°C on a reverse phase (RP) C18 ACQUITY UPLC column (2.1 mm I.D.; 100 mm length; 1.7 µm particle size). The mobile phase was a binary solvent system consisting of phase A (water with 2% acetic acid) and phase B (acetonitrile with 2% acetic acid) with a flowrate of 0.5 mL min^{-1}. The gradient used for the separation was as follows: 0 min 15% B, 3.30 min 20% B, 3.86 min 30% B, 5.05 min 40% B, 5.35 min 55% B, 5.64 min 60% B, 5.94 min 95% B. Following each analysis run, the column was subsequently washed with 100% B for 3 min and equilibrated with 0% B for 3 min.

ANTIOXIDANT ACTIVITY

Reducing power

The Ferric Reducing Power of the extracts was measured [29] using ferric chloride. The absorbance was then measured at 700 nm and the reducing power was expressed as mg gallic acid equivalents (GAE) per 100 g of DM.

DPPH Assay

The antioxidant capacity against 1-Diphenyl-2-picryl-hydrazyl (DPPH) was performed [30]. An aliquot of the appropriate dilution of the extract (1.5 mL) was added to a 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) solution (1.5 mL) and kept in the dark for 60 min. The absorbance was measured at 515 nm and the antiradical activity was expressed as mg gallic acid equivalents (GAE) per 100 g of DM.

ABTS Assay

The radical scavenging power of the extracts was determined by the 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) method [31]. ABTS radical action (ABTS+) stock solution was produced by the reaction of 7 mmol/L of ABTS solution in 2.45 mmol/L potassium persulfate (final concentration) in the dark for 16h. The solution was diluted with ethanol by adjusting the absorbance to 0.700±0.020 at 734 nm. 1000 µl of the diluted extract and 1000 µl of diluted ABTS+ solution was added. The solution was agitated with a vortex mixer. The absorbance was measured after 2.50 min at 734 nm. The results were expressed as mg Trolox eqivalents (TE) per 100 g of DM.

Determination of the Relative Index of Antioxidant Capacity (RACI).

The values of the Antioxidant Capacity Index (RACI) represent the mean scores of the antioxidant activities of each sample, are calculated [32].

STATISTICAL ANALYSIS

The results were subjected to analysis of variance using the Statistica 5.0 package (StatSoft'97 edition) using the least significant difference (Newman–Keuls) test. Significance was defined at ($p < 0.05$).

3. RESULTS AND DISCUSSION

MATURITY INDEX

Olive colour intensity, which can be expressed by the maturity index, varies from green to black. As shown in Table I, the maturity index values increased significantly with the ripening degree. At the end of the ripening process the fruit becomes purple or black due to the accumulation of anthocyanins [33]. The colour of the fruit serves as a common marker for the level of ripening.

Table I - Evolution of maturity index, fruit weight, oil content, moisture and phenolic contents parameters during olive ripening of *Chemlal* and *Blanquette de Guelma* olive fruits

	Sample	MI	Fruit weight (g)	Oïl content (%)	Moisture (%)	Total phenolic content (mg GAE/100 g DM)	O-diphenol (mg CAE/100 g DM)
Chemlal	C_1 (green)	1.37±0.01(b)	1.20±0.03(a)	27.37±0.46(a)	47.00±0.24(e)	15798.34±20.24(f)	6914.18±16.15(e)
	C_2 (spotted)	2.52±0.01(d)	1.31±0.0.5(b)	30.49±0.13(b)	46.66±0.48(e)	12888.01±34.70(e)	7678.64±82.42(f)
	C_3 (purple)	3.25±0.01(e)	1.60±0.04 (e)	34.37±0.31(c)	40.54±0.12(b)	9796.29±76.00(c)	1568.21±22.73(b)
	C_4 (black)	4.71±0.01(g)	1.65± 0.00(f)	35.48±0.34(d)	40.88±0.10(b)	6301.51±9.61(a)	867.6±13.78(a)
Blanquette de Guelma	B_1 (green)	1.13±0.01(a)	1.54±0.01(d)	38.54±0.24(e)	45.62±0.17(d)	22258.56±6.03(g)	6752.06±35.16(d)
	B_2 (spotted)	2.24±0.01(c)	1.50±0.01(c)	42.6±0.41(f)	42.72±0.24(c)	29688.06±68.08(h)	8987.32±4.44(g)
	B_3 (purple)	3.43±0.01(f)	1.92±0.00(h)	46.47±0.10(g)	40.00±0.05(b)	11273.98±81.44(d)	9375.73±40.24(h)
	B_4 (black)	4.86±0.01(h)	1.85±0.01(g)	51±0.35(h)	37.96±0.35(a)	8469.12±45.29(b)	4936.28±46.27(c)

Means ± standard deviation (n = 3). ($^{a-h}$): Means in the column followed by different letters are significantly different ($p<0.05$).

FRUIT WEIGHT

The statistical analysis showed that the olive weight is influenced by the maturity stage and the variety (Tab. I). It was observed that it increased from 1.20 to 1.65 g and from 1.50 to 1.91 g for *Chemlal* and *Blanquette de Guelma* cultivars, respectively. The increasing of weight during ripening can be explained by the increasing of dry matter during the maturation process [34].

MOISTURE CONTENT

The Moisture content of the fruit was significantly influenced by the variety and the ripening degree (Tab. I). The moisture rates decreased through the maturation process from 47.00 to 40.88% and from 45.62% to 37.96% for *Chemlal* and *Blanquette de Guelma* cultivars, respectively. Moisture of olives is favourable for biochemical reactions; high yield rates are reported to affect negatively the oil yield [34]. The obtained results agree with those of Tovar *et al*. [35], who reported that the olive maturation is accompanied by a decrease in the moisture content. Otherwise, the fluctuations of values are correlated to the thickness and permeability of the epicarp characteristics for each variety [36].

OIL CONTENT

The oil content of the olive fruit, expressed as a percentage of dry matter, generally increased during ripening, as shown in Table I. It increased from 27.37 to 35.48%/DM (dry matter), corresponding respectively to the first and the fourth harvest date in the *Chemlal* cultivar, and from 38.54 to 51%/DM in *Blanquette de Guelma*.

Those results indicate an increase in oil content estimated at 22.85% and 24.85% in the two varieties, respectively. An increase from 41.86 to 51.88% in oil content of Tunisian olive cultivars was reported [34]. Significant differences in the olive oil yield were observed during maturity process (for the two cultivars). The statistical analysis showed that the olive oil yield is influenced by the two studied factors (maturity and variety). These results were in agreement with those of other authors [6, 21, 37].

The increasing evolution of oil content in olives during ripening can be explained by lipid biosynthesis during ripening of olives [14]. The oil content generally increased during early fruit ripening until it reached a maximum at mid-maturity and declines slightly as the fruit becomes ripe [6, 38].

PHENOLICS COMPOUNDS AND ORTHO-DIPHENOLS

The total phenolic and *Ortho*-diphenol contents of olive pulp extracts at different stages of maturation of the two cultivars in study are reported in Table I. The statistical study showed significant differences ($p<0.05$) between cultivars and maturation degrees of the two cultivars.

The total phenols content decreases continually from the green to the black maturation stages for *chemlal* (60% of reduction) as reported above [39]. However, the *Ortho*-diphenols content registered the maximum score (7670 mg GAE per 100 g) at spotted maturation stage. This value decreases (88.7%) from the spotted to the black stages.

Total phenols content increase in *Blanquette de Guelma* from the green to the spotted stages (75%); after that, it decreases (75%) from spotted to black stages. *Ortho*-diphenols content increases from green to purple stages (28%) to reach a maximum value of 9376 mg CAE/100 g DM in purple olives and reduced to 4936 mg CAE/100 g in black olives. The results obtained confirmed previous data [35, 40], which reported a decrease in phenolic content from the purple stage to the black one due to the lower PAL activity (L. phenylalanine ammonia-lyase) responsible of the synthesis of phenolic compounds including *ortho*-diphenols during the maturation of olives. Nevertheless, the decrease in phenolic compounds can be related to the increase of the polyphenoloxidase during maturation process [40]. According to Gimeno et al. [41], the polyphenol contents gradually decreases during the ripening process.

PHENOLIC COMPOUNDS PROFILE BY UPLC-PDA

The results of the analysis of phenolic compounds of the two studied varieties of olives at different stages of maturity are shown in Table II. It appears clearly that olive cultivar and ripening degree had a significant influence on the phenolic content.

The analysis of chromatograms of phenolic fractions of different olive samples shows a qualitative and quantitative difference between the two studied varieties. Twelve compounds are identified in the olive extracts of *Chemlal* variety (caffeic acid, vanillic acid, homovanillic acid, *p*-coumaric acid, o-coumaric acid, m-coumaric acid, oleuropein, hydroxytyrosol, rutin, quercetin, luteolin and luteolin-7-glucoside) and sixteen compounds in the extracts of *Blanquette de Guelma* variety (tyrosol, iso-vanillic acid, caffeic acid, vanillic acid, homovanillic acid, *p*-coumaric acid, *o*-coumaric acid, *m*-coumaric acid, sinapic acid, ferulic acid, oleuropein, hydroxytyrosol, rutin, quercetin, luteolin and luteolin-7-glucoside). Tyrosol, ferulic acid, sinapic acid, isovanillic acid were detected only in *Blanquette de Guelma* variety.

The oleuropein is the dominant compound of olive secoiridoides as reported above [42]; this compound is responsible for the olive bitterness [43].

The contents in oleuropein show a net decrease even if with different kinetics during maturation of *Chemlal* and *Blanquette de Gulema* varieties. This result is in accordance with observations of [13, 37]. The decrease in oleuropein content with the progress of

maturation is associated with the increase in esterase and β-glucosidase activities which cause hydrolysis of secoiridoides [10, 11].

The trend of evolution of hydroxytyrosol varies differently between the two studied varieties; its concentration increases during olives ripening of the *Chemlal* variety (+17%), giving an inverse correlation between hydroxytyrosol and oleuropein content; which is in accrdance with the findings of Gomez Rico *et al*. [42]. In *Blanquette de Guelma* variety, hydroxytyrosol content decreased by –31% from the first to the last stage of ripening as described above [39]. The decrease of hydroxytyrosol in olives may be a consequence of hydrolysis and oxidation processes occurring through the olive maturation [39]. This different evolution of hydroxytyrosol could be related to the variety. Bouaziz et al. [18] reported that the hydroxytyrosol concentration of the *Chemlali* olive cultivar increased during maturation. Conversely, Morelló et al. [19] investigating the changes in phenolic compounds during ripening (September to November) of the Arbequina, Farga and Morrut cultivars, noted that the levels of hydroxyrosol decreased.

Four flavonoids are present in all studied olive samples. The flavonoids determined in the two varieties at four stages of maturation are quercetin, luteolin, rutin, and luteolin-7-glucoside. Quercetin constitutes the principal flavonoid of the four maturation stages of *Chemlal*. Luteolin concentration decreases drastically through maturation in the two studied varieties. This decrease is the result of hydrolysis of glucosides [44].

Rutin and luteolin-7-glucoside are present in lowest contents in comparison to quercetin and luteolin ones. Luteolin-7-glucoside content diminishes during olives maturation of both studied varieties. Whereas, rutin content increase, in *Chemlal,* from the first to the second stage of ripening and then decrease. No correlation is observed between maturity and rutin contents in *Blanquette de Guelma.*

Six (6) phenolic acids are identified in the two varieties: caffeic acid, vanillic acid, homovanillic acid, *p*-coumaric acid, *o*-coumaric acid, and m-coumaric acid. Three (3) phenolic acids (iso-vanillic acid, ferulic acid and sinapic acid) are only identified and quantified in the *Blanquette de Guelma* variety.

The four (4) phenolic acids: homovanillic, m-coumaric acid, *o*-coumaric and the caffeic acid are identified in all the analysed samples and a slight variation in their concentration is noticed during the maturation of olives of both varieties (*Chemlal* and *Blanquette de Guelma*). As reported [45], caffeic acid is used directly in the biosynthesis of anthocyans through the olive maturation; this can explain the slight reduction in this phenolic acid concentration from the first stage olives ripening and the last one of *Chemlal* variety.

The vanillic acid and *p*-coumaric are not identified in all of the analysed samples and their concentrations vary slightly during the olives' maturation. The highest contents are noticed for vanillic acid. This acid is identified in *Chemlal* variety only at the green stage (66 mg/kg) and in the three first stages of the *Blanquette de Guelma* variety (67 to 69 mg/kg).

The lowest concentrations are recorded for *p*-coumaric acid. This compound is identified and quantified in the four stages of *Chemlal*: 15 to 16 mg/kg for the *Chemlal* green and black olives respectively; as well as in the spotted, purple and black olives of the *Blanquette de Guelma* variety: 19, 24 et 24 mg/kg respectively.

The *Blanquette de Guelma* variety is characterized by the presence of other phenolic acids (sinapic acid, ferulic acid and iso-vanillic acid) from which the *Chemlal* variety is deprived. The concentrations of these phenolic acids undergo a little change during

Table II - Phenolic contents (mg / kg) of *Chemlal* and *Blanquette de Guelma* olive fruits at four different ripening stages.

Variety	***Chemlal***				***Blanquette de Guelma***			
Sample	**$C_{1(Green)}$**	**$C_{2(Spotted)}$**	**$C_{3(Purple)}$**	**$C_{4(Black)}$**	**$B_{1(Green)}$**	**$B_{2(Spotted)}$**	**$B_{3(Purple)}$**	**$B_{4(Black)}$**
Tyrosol	-	-	-	-	99.59 (a)	108.56 (b)	122.45 (c)	130.19 (c)
Caffeic acid	145.66 (d)	160.24 (f)	140.12 (c)	136.26 (b)	126.36 (a)	155.13 (e)	172.67 (g)	195.46 (h)
Vanillic acid	65,61 (b)	-	-	-	67.68 (b)	68.34 (b)	69.00 (b)	50.00 (a)
Iso-vanillic acid	-	-	-	-	90.94 (a)	92.17 (a)	90.0 (a)	122.60 (b)
Homovanillic acid	236.64 (e)	172.91(b)	235.79 (e)	205.50 (c)	212.23 (d)	241.96 (e)	134.00 (a)	131.90 (a)
P- coumaric acid	15.33 (a)	15.56 (a)	15.76 (a)	15.88 (a)	-	19.25 (a)	23.65 (a)	24.16 (a)
Ferulic acid	-	-	-	-	120.29 (a)	133.82 (b)	122.82 (a)	170.57 (c)
O-coumaric	45.38 (a)	45.95 (a)	44.67 (a)	45.87 (a)	43.33 (a)	43.44 (a)	39.26 (a)	59.00 (b)
Sinapic acid	-	-	-	-	189.6 (a)	187.57(a)	184.08 (a)	274.69 (b)
Rutin	212.93 (b)	773.29 (f)	394.14 (e)	238.07 (c)	220.1 (b)	238.00 (c)	212.86 (b)	180.0 (a)
Luteolin 7 -Glu	280.61 (d)	52.40 (a)	61.96 (b)	52.15 (a)	705.94 (g)	580.43 (f)	422.28 (e)	225.48(c)
m- coumaric	182.72 (d)	187.16 (d)	144.70 (b)	126.88 (a)	187.25 (d)	158.06 (c)	139.01 (b)	161.00 (c)
Oleuropein	870,02 (e)	818,86 (d)	664,16 (c)	320.00 (a)	897.00 (f)	1271,38 (h)	1107,26 (g)	632,33(b)
Quercetin	300,46 (f)	339,91 (g)	280,89 (d)	228,2 (a)	293,43 (e)	247,58 (b)	252,83 (b)	265,42 (c)
Luteolin	132,164 (d)	49,24 (b)	25,35 (a)	25,89 (a)	354,91 (g)	161,28 (e)	168,42 (f)	125,56 (c)
Hydroxytyrosol	1370.18 (e)	1377.49 (e)	1272.87(d)	1661.03 (g)	1444.75 (f)	1141.04 (c)	1063.23 (b)	998.68 (a)

- : Non detected. ($^{a-h}$): Means in the row followed by different letters are significantly different ($p<0.05$).

maturation. The highest contents are recorded for the sinapic acid. The quantities vary from: 190 to 275 mg/kg for the green and the black stages, respectively. The lowest values are recorded for the iso-vanillic acid. This phenolic acid is quantified in all the analysed samples; its concentration increases through maturation from 91 (green olives) to 123 mg/kg (black olives).
Tyrosol is identified only in *Blanquette de Guelma*, its content increases during maturation from 99 mg/Kg to 130 mg/Kg dissimilar to Yorulmaz *et al*. [44] finding.

ANTIOXIDANT ACTIVITY

The antioxidant activity trough the maturation process of the studied varieties is assessed by measuring reducing power, the antiradical activity toward DPPH and ABTS radicals (Tab. III).

REDUCING POWER

The analysis of the reducing power of the olive samples (Tab. III) shows that variety and maturation stage have a significant effect ($p<0.05$), which diminishes gradually during maturation and this, for both varieties. The extracts of *Blanquette de Guelma* exert, at the four stages, the highest reducing powers in comparison to *Chemlal* ones due to the high contents of some reducing compounds (Content of luteolin and tyrosol) in this variety and probably to the presence of more phenolic acids (ferulic acid, sinapic acid and iso-vanillic acid). It is reported that hydroxycinnamic acids exert a strong antioxidant activity [46]. Those reducing compounds cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form [47].
A loss in reducing power through maturation appears from the obtained results, which is plausible due to losses in phenolic compounds with an antioxidant potential as oleuropein, hydroxytyrosol, luteolin and rutin.
The reduction in the reducing power for *Chemlal* (64. 65%) from the first to the last ripening stage is more pronounced than in *Blanquette de Guelma* (22.4%) probably due to the slight modification in quercetin and luteolin contents in this variety and to the slow maturation process of this variety. A decrease of 66% in reducing capacity through sampling was reported [48].
Several authors [39, 49] have shown that the reducing power of an extract is mainly due to phenolic compounds. This is confirmed by the high significant positive correlations between the reducing potential of the olive extracts and the contents in polyphenols. The correlation coefficients are of 0.985 and 0.747 for *Chemlal* and *Blanquette de Guelma,* respectively. Significant correlations are also established between reducing power and *ortho*-diphenol content of the two varieties, respectively.

ANTIRADICAL ACTIVITY (ANTIRADICAL DPPH)

The obtained results (Tab. III) showed that Blquette de Guelma is more efficient than Chemlal at the four stages that have been studied. A different evolution of the antiradical activity against the radical DPPH is observed for the two varieties. Antiradical activity reduces continually through maturation in *Chemlal* of about 36% from the green to the black maturation stages. In contrast, this activity increases in *Blanquette de Guelma* by 9% from green to the spotted stages, which is closely related to the increase of *ortho*-diphenolic compounds as oleuropein (50%). Roche *et al*. [50] classified phenolic compounds of olives in terms of reaction rate with DPPH radical as: Hydroxytyrosol>oleuropein>caffeic acid. In fact, significant positive correlations ($p<0.05$) are noticed between this activity and their contents in total polyphenols ($r = 0.921$ for *Chemlal*, and $r = 0.706$ for *Blanquette de Guelma*) and *ortho*-diphenols ($r = 0.754$ for *Chemlal*, and $r = 0.690$ for *Blanquette de Guelma*). In addition, the slight modifications in quercetin, luteolin and caffeic acid content in this variety maximize the radical scavenging capacity due to the presence of 3 and 5-hydroxyl groups and the *O*-dihydroxy structure. Indeed, the importance of luteolin in the observed effects was noted [50].
These results agree with those of other authors [48, 51] of which the green olives extract rich in phenolic compounds exert the best scavenger activities on the DPPH radical.

Table III - Antioxidant activities of olives extracts of *Chemlal* and *Blanquette de Guelma* at four different ripening stages.

Variety	Sample	RP (mg GAE / 100 g DM)	DPPH (mg GAE / 100 g DM)	ABTS (mg TE / 100 g DM)
Chemlal	C_1 (green)	19347.93±120.73(d)	27056.97±80.33(d)	1193.25±5.49(g)
	C_2 (Green-Spotted)	14391.72±140.70(c)	25918.60±90.22(c)	699.69±2.71(c)
	C_3 (purple)	9648.41±106.36(b)	25223.25±35.28(b)	627.24±4.76(b)
	C_4 (black)	6855.23±78.04(a)	20103.48±100.62(a)	485.09±3.27(a)
Blanquette de Guelma	B_1 (green)	24440.38±96.72(g)	28398.83±89.68(e)	1093.28±7.51(f)
	B_2 (Green-spotted)	22635.03±25.54(f)	31074.41±99.71(g)	1354.98±6.00(h)
	B_3 (purple)	20906.32±140.70(e)	28369.76±92.44(e)	959.17±3.75(e)
	B_4 (black)	18964.72±39.02(d)	29151.16±111.21(f)	769.11±14.50(d)

Means ± standard deviation (n = 3). ($^{a-h}$) Means in the column followed by different letters are significantly different ($p<0.05$).

ANTIRADICAL ACTIVITY (RADICAL ABTS)

All of the analysed olive samples have the capacity to trap the ABTS radical with significant differences ($p<0.05$) according to the variety and the degree of maturity.

The green olive extracts of the *Chemlal* variety (1193 mg TE per 100 g DM) and the spotted ones of the *Blanquette de Guelma* variety (1355 mg TE per 100 g DM) exert the best scavenger activities on the ABTS radical. These samples are characterised by the highest contents in total polyphenols. By contrast, black olive extracts with low contents are the least efficient (485 mg TE per 100 g DM and 769 mg TE per 100 g DM, respectively).

The relation between the contents in polyphenols and the antioxidant powers are largely studied in olives [46, 52, 53]. Significant positive correlations are noticed between the scavenger activity on the ABTS radical and the contents in total polyphenols ($r = 0.908$for *Chemlal* and $r = 0.967$ for *Blanquette de Guelma*) and *ortho*-diphenols ($r = 0.684$ for *Chemlal* and $r = 0.635$ for *Blanquette de Guelma*).

RELATIVE INDEX OF ANTIOXIDANT CAPACITY (RACI)

The RACI is validated as a reference to classify the samples according to their antioxidant potential that results from the combination of all the methods used to study the antioxidant activity because this index makes the comparison of the data more reliable.

The results (Fig. 1) show that the green and spotted olives of the two varieties *Chemlal* and *Blanquette de Guelma* give the highest indices RACI. The extracts of the green olives of *Chemlal* and spotted ones of *Blanquette de Guelma* mark the superiority in their contribution to the totality of the tests, mentioning the RACIs of +1.339 and +1.187, respectively. Whereas, the purple and black stages of the two studied varieties note the lowest RACI values.

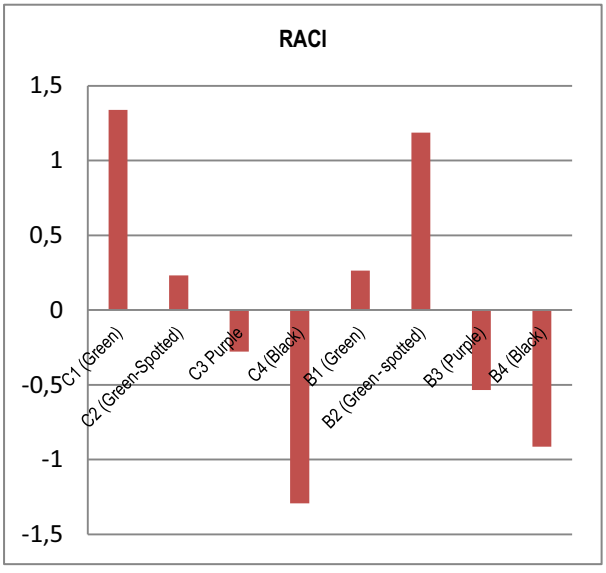

Figure 1 - Relative antioxidant capacity index (RACI) of *Chemlal* (C) and *Blanquette de Guelma* (B) during ripening. Indices 1, 2, 3 and 4 Correspond to date of harvest

4. CONCLUSION

The process of maturation of *Chemlal* and *Blanquette de Guelma* varieties affects considerably the phenolic composition and antioxidant activity. The green stage maturity of *Chemlal* and the spotted one of *Blanquette de Guelma* have the highest phenol content. Hydroxytyrosol concentration diminishes through maturation in parallel to the diminution of oleuropein content.Tyrosol, ferulic acid, sinapic acid, isovanillic acid are considered as varietal factors since they were detected only in *Blanquette de Guelma*.

Phenolic compounds affect the antioxidant activity of olive extracts. A significant decrease in the antioxidant activity of the olive fruits is observed through maturation. The loss in reducing power and antiradical activities is more pronounced in *Chemlal*. Significant correlations are established between antioxidant activities and phenolic and *ortho*-diphenols contents; testifying that antioxidant activity of olives is also influenced by the phenolic content and the phenolic profile.

Results obtained allow us to better estimate the optimum harvest time of the Algerian olive cultivars of *Chemlal* and *Blanquette de Guelma* in order to improve the quality and the bioactivity of olive oils extracted. Towards data obtained in the phenolic composition and antioxidant capacity the harvest can occur when the olives are in the spotted stage, corresponding to mid-December. This period allows a good combination between the phenolic content and antioxidant activity.

Further studies are requested in order to completely understand the full impact of maturation on the olive fruits composition.

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Conflicts of interest

There are no conflicts of interest to declare.

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