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Original Article

Phenols and antioxidant properties of different parts of Tunisian globe artichoke heads

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Abstract

This study aimed to evaluate the variation of biochemical properties from the different parts (outer, intermediate and inner bracts and receptacle) obtained from capitulum (head) of two globe artichokes cultivars ('Violet d'Hyères' and 'Blanc d'Oran') grown in Tunisia. We determined the total phenols, o-diphenols, flavonoids, anthocyanins and tannins compounds, as well as antioxidant capacity measured by three different methods: DPPH, ABTS and reducing power assay. The highest contents of phenols and O-diphenols were found in 'Violet d'Hyères' whereas the lowest anthocyanins and flavonoids levels were noticed in 'Blanc d'Oran'. Significant differences were observed with respect to the head's part where inner bracts exhibited the highest contents of phenols (2395.5 and 1537.8 mg kg^{-1}, respectively for 'Violet d'Hyères' and 'Blanc d'Oran'), o-diphenols (572.2 and 230.7 mg kg^{-1}, respectively for 'Violet d'Hyères' and 'Blanc d'Oran'), flavonoids (272.8 and 214.8 mg kg^{-1}, respectively for 'Violet d'Hyères' and 'Blanc d'Oran'), tannins (505.9 and 553.9 mg kg^{-1}, respectively for 'Violet d'Hyères' and 'Blanc d'Oran') and anthocyanins (442.4 and 639.6 mg kg^{-1}, respectively for 'Violet d'Hyères' and 'Blanc d'Oran'). Receptacle showed the lowest contents of all parameters under study. The antioxidant assays reflected the highly diversified composition of the head's part. In conclusion, the high antioxidant activity and phenolic contents found in the globe artichocke bracts, suggested to use them as possible ingredients for functional foodstuffs.

Mots clés :
Globe d'artichauts
Bractées
Réceptacle
Teneurs en phénols
Activité antioxydante

Résumé

Teneurs en phénols et propriétés antioxydantes de différentes parties des globes d'artichauts de la Tunisie. L'objectif de cette étude était d'évaluer la variation des propriétés biochimiques des différentes parties (bractées extérieures, intermédiaire et interne et réceptacle) obtenus à partir de la capitule de deux cultivars de globe d'artichauts ('Violet d'Hyères' et 'Blanc d'Oran') cultivés en Tunisie. Nous avons déterminé les teneurs en phénols totaux, o-diphénols, flavonoïdes, anthocyanes et tanins condensés, ainsi que la capacité antioxydante mesurée par trois méthodes différentes: DPPH, ABTS et le pouvoir réducteur. Les teneurs les plus élevées en phénols et en *o*-diphénols ont été trouvés dans 'Violet d'Hyères' tandis que les plus faibles teneurs en anthocyanes et en flavonoïdes ont été remarqués dans 'Blanc d'Oran'. Des différences significatives ont été

observées par rapport à la partie de la capitule où les bractées intérieures présentent les teneurs les plus élevées en phénols (2395,5 et 1537,8 mg kg^{-1}, respectivement pour 'Violet d'Hyères' et 'Blanc d'Oran'), o-diphénols (572,2 et 230,7 mg kg^{-1}, respectivement pour 'Violet d'Hyères' et 'Blanc d'Oran'), flavonoïdes (272,8 et 214,8 mg kg^{-1}, respectivement pour 'Violet d'Hyères' et 'Blanc d'Oran'), tanins (505,9 et 553,9 mg kg^{-1}, respectivement pour 'Violet d'Hyères' et 'Blanc d'Oran') et anthocyanes (442,4 et 639,6 mg kg^{-1}, respectivement pour 'Violet d'Hyères' et 'Blanc d'Oran'). Le réceptacle a montré les contenus les plus faibles de tous les paramètres étudiés. Les tests antioxydants reflètent la composition très diversifiée des différentes parties du globe d'artichaut. En conclusion, l'activité antioxydante élevée et les teneurs élevés en composés phénoliques trouvés dans les bractées de globe d'artichaut, ont suggéré de les utiliser comme ingrédients possibles pour les produits alimentaires fonctionnels.

INTRODUCTION

Cynara cardunculus L. var. *scolymus* (L.) Fiori, popularly known as artichoke, is an ancient herbaceous perennial plant, originating from the Mediterranean area, which is widely cultivated today (Bianco, 2005). Its annual global production of approximately 1.6 million metric tonnes (t) is dominated by Southern Europe with Italy and Spain being the world's leading producers. The globe artichoke is also cultivated in the Near East, North Africa, South America, United States and recently its cultivation has been spreading in China (12 kha) and Peru (8 kha). Tunisia is one of the great producers of globe artichoke where it is grown on 2750 ha and it constitutes an important crop, especially in the Low Valley of Medjerda, with 18 000 t (Faostat, 2013).

The edible part of the artichoke plant include the enlarged receptacle and the tender thickened bracts bases ('heart') of the head (capitulum), which is the immature inflorescence (Lombardo *et al*., 2013), consumed as a fresh, canned or frozen worldwide. The tender inner parts constitute nearly 35–55% of the fresh weight of the head, depending on the variety and the harvesting time (Lattanzio *et al*., 2009). The globe artichoke is an attractive source of natural antioxidants since it is rich in polyphenols mainly phenolic acids and flavonoids (Pandino *et al*., 2012a). Regarding heads, young globe artichoke heads were reported to contain more antioxidant phenolic compounds than mature ones (Wang *et al*., 2003); a different concentration of phenolics was also observed in globe artichoke head harvested from November to April, revealing the influence of harvest time on phenolics composition (Pandino *et al*., 2013). Moreover, the different parts of the heads have been little investigated (Romani *et al*., 2006; Fratianni *et al*., 2007). In this report, the objective of the present study was to compare biochemical properties from the different parts (outer, intermediate and inner bracts and receptacle) obtained from capitulum (head) of two globe artichokes cultivars ('Violet d'Hyères' and 'Blanc d'Oran') grown in Tunisia.

MATERIALS AND METHODS

Plant Material, Management Practices and Capitula Sampling

The field experiment was conducted at the experimental station in the Technical Center of Potato and Artichoke of Tunisia located in Mannouba (north of Tunisia), on Mannouba Plain, a typical area for globe artichoke cultivation in Tunisia (latitude 36°49'25.24" N, longitude 9°57'55.09"W, altitude 595 m). Two cultivars of globe artichoke at maturity stage were considered, 'Blanc d'Oran' and 'Violet d'Hyères'. The field trials were carried out during the 2011-2012 season. Crop management practices were subsequently performed according to the local practices. At least five disease-free capitula per replicate were harvested at the usual marketing stage, when the floral bud was ≤2 mm (Mauromicale and Ierna, 2000). The bracts were manually removed, separated into 'outer bracts', 'intermediate bracts', 'inner bracts (remain bracts)' and the receptacle, washed with tap water and, then, subjected to chemical characterization.

Total Phenols and o-Diphenols

An amount (5 g) of blended fresh sample was treated with 20 ml of methanol 80% containing 1%

hydrochloric acid, homogenized for 2 min in an Ultra-Turrax T25 (Janke and Kunkel Ika-Labortechnik, Staufen, Germany) and stirred at the room temperature for 1 h. Phenols and *o*-diphenols contents were estimated colorimetrically at 765 nm using the Folin–Ciocalteu reagent (Montedoro *et al*., 1992) and the results were expressed as mg hydroxytyrosol equivalents per kg of fresh weight (FW).

Total flavonoids

An aliquot (250µL) of each methanolic extract or standard solution was mixed with 1.25 ml of dH_2O and 75µL of 5% $NaNO_2$ solution. After 6min, 150µL of 10% $AlCl_3$ solution was added. Five minutes later, 0.5 ml of 1M NaOH solution was added and then the total volume was made up to 2.5 ml with dH_2O. Following, the complete mixing of the solution, the absorbance against blank was determined at 510 nm (Zhishen *et al*., 1999). Total flavonoid contents were expressed as mg catechin equivalents per kg of FW.

Condensed tannins

Condensed tannins were determined according to the method of Julkunen-Titto (1985). An aliquot (50 µL) of each methanolic extract or standard solution was mixed with 1.5 ml of 4% vanillin (prepared with MeOH), then 750 µL of HCl (12M) was added. The well-mixed solution was incubated in the dark at ambient temperature for 20 min. The absorbance against blank was read at 500 nm. The results were expressed as mg catechin equivalents per kg of FW.

Total anthocyanins

The fresh matter (1 g) was extracted with 25 mg/ml of acidified methanol (1% HCl) for 2 h at room temperature in the dark and, then, centrifuged at 450 × g for 15 min. Anthocyanin levels were calculated from the methanolic extract as $A_{530} - (0.24 \times A_{653})$ and total anthocyanin content was expressed as mg cyaniding 3-glucoside equivalents per kg of FW, using an extinction coefficient of 26.900 L mol^{-1} cm^{-1} at 530 nm and a molar mass of 449.2 g mol^{-1} (Giusti and Wrolstad, 2000).

DPPH assay

20-µL from the stock solution of each extract, used for total polyphenol assay, were dissolved in absolute ethanol to a final volume of 1 ml and then added to 1 ml DPPH (0.1 mM, in absolute ethanol). The reaction mixture was kept at the room temperature. The optical density (OD) of the solution was measured at 517 nm after 20 min. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. Inhibition of the free radical DPPH as a percentage was calculated (Kontogiorgis and Hadjipavlou-Litina, 2003).

ABTS assay

A stock solution of 7 mM of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) aqueous solution was prepared. ABTS radical cations (ABTS*+) were produced by the reaction of the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) (Re *et al*., 1999) after incubation in the dark at the room temperature for 16 h. Finally, the stock solution was diluted with ethanol to obtain an absorbance of 0.7±0.02 at 734 nm and equilibrated at 30°C. A reagent blank reading was taken (A0). For the spectrophotometric assay, 3.9 ml of the ABTS*+ diluted solution was mixed with 100 ml of phenolic extract or trolox. Mixtures were mixed vigorously for 30 s and were allowed to stand for 5 min in the dark at the room temperature. Subsequently, the absorbance for each sample (ABTS*+ solution plus compound, At) was measured at 734 nm and corrected for the absorbance of a control (ABTS*+ solution without test sample, A0). The radical-scavenging activity of the samples was expressed as mmol Trolox equivalent per kg (Dabbou *et al*., 2010).

Reducing power property

In the reducing power assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each extract. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} concentration can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. An aliquot of each phenolic extract (250 µL) was mixed with 250 µL of sodium phosphate buffer (0.2 M, pH 6.6) and 250 µL of 1% $K_3Fe(CN)_6$ incubated at 50 °C for 20 min. After adding 250 µL of 10% trichloroacetic acid, the mixture was centrifuged at 3750g for 10 min. The supernatant (100 µL) was then taken out and immediately mixed with 100 µL of MeOH and 25 µL of 0.1% ferric chloride. After incubation for 10 min, the absorbance against blank was determined at 700 nm (Oyaizu, 1986). Increased absorbance indicated an increased reducing power.

Statistical Analysis

Significant differences between varieties (p<0.05) were determined by Students t-test whereas differences between globe artichoke components were determined by Duncan test, using the SPSS program, release 17.0 for Windows (SPSS, Chicago, IL, USA). All data presented represent mean values of three independent experiments (n=3).

RESULTS AND DISCUSSION

Phenolic Compounds

Total Phenols, flavonoids, *o*-diphenols, tannins and anthocyanins contents of the different parts of heads (outer, intermediate, and inner bracts and receptacle) of the two cultivars of globe artichokes grown in Tunisia are shown in Table 1.

Table 1. Chemical composition (mg kg^{-1} FW) in heads of the two studied cultivars of globe artichoke grown in Tunisia

	Outer Bracts	Intermediate Bracts	Inner Bracts	Receptacle
		Violet d'Hyères		
Phenols	1243.1±8.0 ^{b}	1004.9±52.9 ^{c}	2395.5±14.4 ^{a}	730±41.1^{d}
***O*-diphenols**	187.4±11.7 ^{c}	269.4±29.7 ^{b}	572.2±5.8 ^{a}	154.1±4.9^{c}
Flavonoids	251.7±8.5 ^{a}	188.5±22.8 ^{b}	272.8±13.6 ^{a}	122.3±9.6^{c}
Anthocyanins	54±9.5 ^{c}	188.4±25.8 ^{b}	442.4±49.4 ^{a}	24.6±0. 9^{c}
Tannins	228.9 ± 20.9 ^{b}	174 ± 10.5 ^{b}	505.9 ± 57.6 ^{a}	171.7±13.7^{b}
		Blanc d'Oran		
Phenols	1024.3±130.1 ^{x}	772.6±21.6 ^{y}	1537.8±118.2 ^{w}	595.9±54.2^{z}
***O*-diphenols**	211.4±2.3 ^{x}	180.4±9.7 ^{y}	230.7±10.5 ^{w}	73.6±11.7^{z}
Flavonoids	163.6±23.4 ^{x}	151.6±1.7 ^{x}	214.8±11.1 ^{w}	87.7±6.8 ^{y}
Anthocyanins	24.7±1.4 ^{z}	66.2±8.1 ^{x}	639.6±5.8 ^{w}	36.8±7.6^{y}
Tannins	141.9 ± 21 ^{x}	146.5 ± 14.3 ^{x}	553.9 ± 57.1 ^{w}	139.6±10.4 ^{x}

Mean composition of sampled artichoke from three replications ± standard deviation. Different letters (a–d) and (w-z), for the same parameter, within columns indicate significant differences (p < 0.05) with respect to cultivar.

The fresh artichoke contained noticeable amounts of total phenol contents. The higher concentration was present in the inner bract followed by outer and intermediate bracts and receptacle for both cultivars, confirming the findings of Pandino *et al*. (2012a) and Fratianni *et al*. (2007). *O*-diphenols concentrations were also studied and significant differences were found in their contents. Similarly to the total phenol concentration, the highest amount of *o*-diphenols was observed in inner bracts of both cultivars, whereas the lowest amount in receptacle of 'Violet d'Hyères' (154.1 mg kg^{-1} FW) and in outer bracts of 'Blanc d'Oran' (73.6 mg kg^{-1} FW).

Flavonoids which are the largest group of plant polyphenols were very abundant in the head of globe artichoke with the higher quantity in the inner bracts of both cultivars (272.8 mg kg^{-1} and 214.8 mg kg^{-1} FW for 'Violet d'Hyères' and 'Blanc d'Oran', respectively). These results were in agreement with the results of Romani *et al*. (2006), who found a higher quantity of flavonoids in head of "Violetto di Toscana" variety and with those of Lombardo *et al*. (2009), who mentioned a higher quantity of flavonoids in a whole globe artichoke head for all the genotypes of globe artichoke under study.

Concerning anthocyanins contents, previous works showed that anthocyanin pigments were present only in heads in form of glucosides and sophorosides and were responsible for the color of artichoke capitula, ranging from green to violet (Lattanzio *et al*., 2009). These compounds were significantly different between the different parts of heads (Table 1). Inner bracts contained the highest quantities of anthocyanins (442.4 and 639.6 mg kg^{-1} FW for 'Violet d'Hyères' and 'Blanc d'Oran', respectively). However, the lowest amounts were observed in the receptacle for 'Violet d'Hyères' (24.6 mg kg^{-1} FW) and in outer bracts for 'Blanc d'Oran' (24.7 mg kg^{-1} FW).

Regarding the tannins, the major amount was found in inner bracts of both cultivars (505.9 and 553.9 mg kg^{-1} FW for 'Violet d'Hyères' and 'Blanc d'Oran', respectively). In addition, significant differences were observed among the different parts of the head for both cultivars.

DPPH assay

The DPPH scavenging activities of the different parts of heads are shown in Figure 1. The different parts of the head showed potent free radical scavenging activity on DPPH. Comparing the two cultivars, a

major significant difference (p<0.05) was observed for the inner bracts and receptacle.

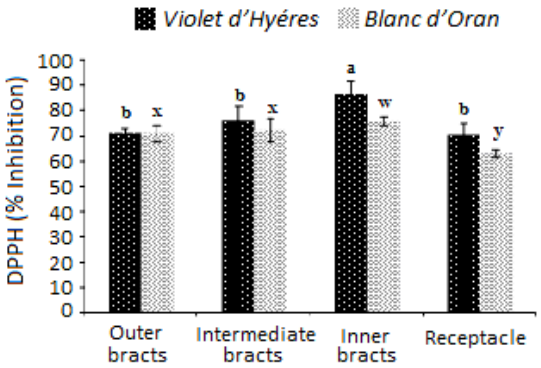

Figure 1. Radical scavenging activity determined by DPPH assay of the different parts of the heads of globe artichokes. Vertical bars represent the standard deviation.

The inner bracts showed the highest DPPH radical scavenging activity followed by intermediate, outer and receptacle, as found for the phenolic content. These results are in agreement with previous studies (Tabart *et al*., 2009; Dabbou *et al*., 2010) which suggested that mainly these compounds are responsible for the antioxidant activity.

ABTS assay

The $ABTS^{+}$ scavenging abilities of the different parts of heads are plotted in Figure 2. When comparing the two cultivars, a major significant difference was observed for outer, intermediate bracts and receptacle. In 'Violet d'Hyères', the outer bracts (0.58 mmol trolox kg^{-1}) and receptacle (0.56 mmol trolox kg^{-1}) contained the higher ABTS values respect to other bracts. In 'Blanc d'Oran' the higher quantity was observed in the intermediate bracts (0.56 mmol trolox kg^{-1}).

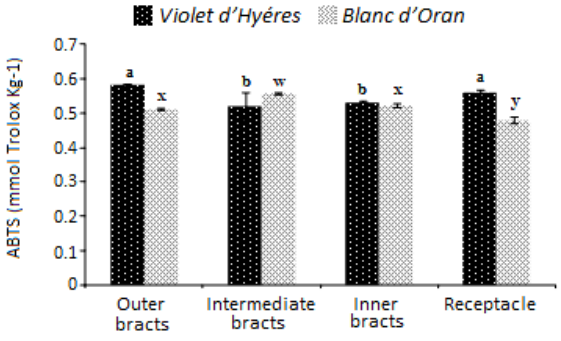

Figure 2. Radical scavenging activity determined by ABTS assay of the different parts of the heads of globe artichokes. Vertical bars represent the standard deviation.

Reducing Power

Reducing power is associated with antioxidant activity and may serve as significant reflection of the antioxidant activity. The results of this research showed that the higher reducing power (as indicated by the absorbance at 700 nm) was detected for the intermediate, inner bracts and receptacle of 'Blanc d'Oran' (Figure 3). For 'Violet d'Hyères', the receptacle contained the highest reducing power (0.65). Comparing the two cultivars, significant difference was observed for the different parts of the globe artichoke head.

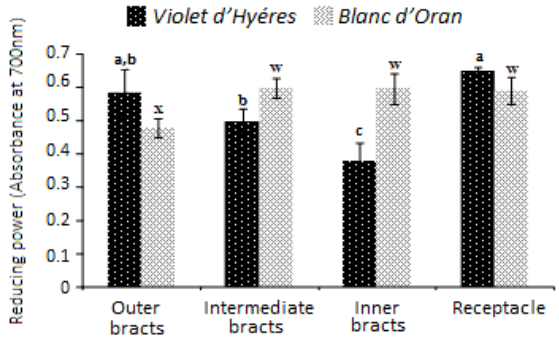

Figure 3. Reducing power of the different parts of the heads of globe artichokes. Vertical bars represent the standard deviation.

CONCLUSION

The data resulting from this research work indicated that 'Violet d'Hyères' cultivar had the highest values of phenolic compounds, confirming the results observed in our previous work in terms of polyphenols profile (Dabbou *et al*., 2015). Our results showed that phenolic content was affected by cultivar and head fraction. The results presented herein indicated that artichoke heads could represent an important source of polyphenols in comparing with the other vegetables because of their ability to supply a high level of these important biomolecules and because they can be used in therapeutic and neutraceutic activity. However, future researches are still necessary to define the combined effect of cultivar, head parts and environmental factors (Lombardo *et al*., 2009; Pandino *et al*., 2012b)

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