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Effect of harvesting time on phenolic compounds and antiradical scavenging activity of *Borago officinalis* seed extracts

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ABSTRACT

Borage seeds (*Borago officinalis* L.) were sampled in Amdoun region (North of Tunisia) during their ripening stage in order to analyse their phenolic compounds and to ascertain their antiradical scavenging activity. The harvesting time effect on some physical properties of borage seed was significant. The increase of dry weight (from 10 to 90%) during ripeness was correlated negatively with that of moisture content (from 90 to 10%). Seed phenolic contents ranged from 2.45 to 10.98 mg GAE/g DW. HPLC analysis permitted to identify nine phenolic acids during seed maturation with the predominance of rosmarinic, syringic and sinapic acids. Total phenolic contents and IC_{50} values in seed during their maturation, allowed to conclude that antioxidant activity does not depend on the high content of total phenolics but on the phenolic composition.

1. Introduction

Recently, borage (Boraginaceae family) has been the subject of increasing agricultural interest because of the potential market for γ-linolenic acid (GLA) (Hamrouni et al., 2002; Mhamdi et al., 2009) and for antioxidant and ROS-scavenging properties of borage meal extract which is attributed to their phenolic constituents (Wettasinghe et al., 2001). The dominant antioxidative compound was identified as rosmarinic acid (Wettasinghe et al., 2001). Nowadays, antioxidants have gained more importance because of their positive involvement as health promoters in conditions such as cardiovascular problems, atheroscle-rosis, treatment of many forms of cancer, and the ageing process (Packer, 1999). Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical scavengers (Duh, 1998) such as rosmarinic acid that a multitude of biological activities have described for this phenolic acid such as astringent, antioxidative, antiinflammatory, antimutagen, antibacterial and antiviral (Parnham and Kesselring, 1985).

The phenolic composition and the antioxidant activity during seed ripening have not yet been studied. Phenolic composition changes during seed ripening stages can affect the human nutritional value and health properties. Hence, this study was undertaken to investigate changes in the phenolic components and antiradical scavenging activity at different stages of seed ripening in order to determine the optimal accumulation period of desirable compounds and to try to valorize this borage seed as source of bioactive molecules. We also aimed at studying whether a correlation exists between phenolic composition and the antioxidant activity at different ripening stages.

2. Materials and methods

2.1. Plant material

Borage seeds used in this study were collected from the region of Amdoun (Beja, North West of Tunisia) at different dates selected on the basis of their ripening stage (Table 1).

2.2. Extraction and determination of total polyphenols

Harvested seed were dried at room temperature for 1 week. Seed extracts were obtained by stirring 1 g of dry seed powder with 10 ml of pure methanol for 30 min. The extracts were then kept for 24 h at 4 °C, filtered through a Whatman no. 4 filter paper, and evaporated under vacuum to dryness and stored at 4 °C until analysed. The content of total phenolic of *B. officinalis* methanolic extract were determined using the Folin–Ciocalteu (F–C) reagent according to the method described by Dewanto et al. (2002) using gallic acid as reference.

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Table 1
Physical characteristics of borage fruit during maturation.

Characteristic	Days after anthesis					
	16	26	36	48	58	78
Fruit colour	Green	Green	Green-brown	Green-brown	Brown	Brown
Dry weight (%)	10 ± 0.14^{d}	11 ± 0.11^{d}	55 ± 0.79^{c}	70 ± 0.13^{b}	85 ± 1.02^{a}	90 ± 0.55^{a}
Moisture content (%)	90 ± 0.42^{a}	89 ± 1.69^{a}	45 ± 0.97^{b}	30 ± 0.66^{c}	15 ± 0.12^{d}	10 ± 1.33^{d}

Values followed by the same small letter did not share significant differences at 5% (Duncan test).

2.3. Hydrolysis and identification of phenolic compounds using HPLC

Dried samples from seeds were hydrolysed according to the method of Proestos et al. (2006), slightly modified. 20 ml of methanol containing BHT (1 g l^{-1}) were added to 0.5 g of a dried sample. Then 10 ml of 1 M HCl were added. The mixture was stirred carefully and sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The obtained mixture was injected to HPLC. The phenolic compounds' analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC, Palo Alto, CA) coupled with an UV–vis multiwavelength detector. The separation was carried out on a 250 × 4.6-mm, 4-μm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 ml min^{-1}. The gradient programme was as follows: 15% A/85% B, 0–12 min; 40% A/60% B, 12–14 min; 60% A/40% B, 14–18 min; 80% A/20% B, 18–20 min; 90% A/10% B, 20–24 min; 100% A, 24–28 min (Bourgou et al., 2008). The injection volume was 20 μl, and peaks were monitored at 280 nm. Samples were filtered through a 0.45 μm membrane filter before injection. Peaks were identified by congruent retention times compared with standards. Analyses were performed by triplicate

2.4. DPPH assay

The electron donation ability of the obtained methanol extracts was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al. (1988). 2 ml of methanolic extracts at different concentrations (borage seed extracts (10 and 50 μg ml^{-1})) were added to 0.5 ml of a 0.2 mmol l^{-1} DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity (three replicates per treatment) was expressed as IC_{50} (mg ml^{-1}), the concentration required to cause a 50% DPPH inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of seed extract (Patro et al., 2005). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect } (\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. BHT was used as a positive control. Samples were analysed in triplicate.

2.5. Statistical analysis

All data were reported as means ± standard deviation of three samples. Statistical analysis was performed by the "STATISTICA v 5.1" software (Statsoft, 1998). Differences were tested for significance by using the ANOVA procedure, using a significance level of $p < 0.05$.

3. Results and discussion

3.1. Physical characteristics of borage seed

The borage seed colour was adopted as a visual ripening criterion. In fact, the seeds turn from green (16 and 26 days after anthesis, DAA) to green-brown (36 and 48 DAA) and finally to brown (58 and 78 DAA) when completely ripe.

In the other hand, the effect of harvesting time on seed dry weight was significant ($p < 0.05$) as shown in Table 1. The seed weight at 16 DAA (10%) was the lowest because of seed immaturity. Seeds increased progressively in weight as growth progressed to obtain a maximum of 90% at ripe seed (78 DAA). These results were similar to those of Hamrouni et al. (2002) who signalled that there is a strong positive correlation between maturity stage and seed dry matter due to the accumulation of organic matter in the ripe seeds.

3.2. Polyphenol content evolution during seed maturation

During maturation, polyphenolic amounts showed an irregular variation during different stages of maturity and reaching a maximum value at full ripening with 10.98 mg GAE/g DW (Table 2). The amount was taken in consideration for raison to valorize the borage seed.

Table 2 showed that the amounts of polyphenols determinate by Folin–Ciocalteu method was highest than those determined by HPLC method. Probably the main cause of the difference obtained by the two methods is the fact that the Folin–Ciocalteu method does not provide a specific assay for phenolic compounds as it reacts positively with many easily oxidizable non-phenolic compounds (Singleton, 1988; Escarpa and Gonzalez, 2001).

As can be seen in Fig. 1, the increase of total phenolic content observed at last stages of seed maturation coincided with the increase of the temperature in the sampling region. Toor et al. (2006) showed that high temperature had a positive effect on the accumulation of major antioxidant components of tomato. So, the accumulation of phenolic compounds was considered as a protective mechanism of plant against environmental conditions.

3.3. Evolution of phenolic acid amounts

In this study, HPLC successfully identified nine phenolic acids during the different maturation seed stages: gallic, sinapic,

Table 2
Polyphenolic contents determined by Folin–Ciocalteu and HPLC during seed maturation.

Days after anthesis	mg GAE/g DW (Fo–Cio)	mg/g MS
16	9.04 ± 0.8^{b}	5.23
26	4.40 ± 065^{d}	4.29
36	2.45 ± 0.34^{e}	1.29
48	3.76 ± 0.67^{de}	1.46
58	7.04 ± 0.92^{c}	3.04
78	10.98 ± 0.97^{a}	8.55

Values followed by the same small letter did not share significant differences at 5% (Duncan test).