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Polyphenols content and antioxidant capacity of thirteen faba bean (*Vicia faba* L.) genotypes cultivated in Tunisia

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ABSTRACT

In vitro antioxidant capacity of thirteen genotypes of faba bean (*Vicia faba* L.) collected during three different growing stages (vegetative, reproductive and maturity) have been evaluated. For the vegetative stage (VS) and reproductive stage (RS) the whole plant (WP) has been used for the analysis while for the maturity stage (MS) the plant was divided and analyzed in different parts, the whole seed (WS), seed coat (SC), cotyledons (Cot) and pod (Pod). The antioxidant capacity has been evaluated by means of the total phenolic and total flavonoid contents (TPC and TFC respectively), the ferric-reducing/antioxidant power (FRAP) and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assays. The results showed different antioxidant properties for the same genotype when considering the different parts or stages of the plant. High content of both total phenolics (>92.85 mg as gallic acid equivalent (GAE)/g of dried plant) and flavonoids contents (>11.87 mg as rutin equivalent (RE)/g of dried plant) were found for all genotypes during the vegetative and the reproductive stages, which also showed the highest antioxidant activity (FRAP value ≥1.157 mmol/g and DPPH radical-scavenging capacity >43.49%), indicating their potential as natural sources of antioxidant foods. For the maturity stage, the highest values for the TPC, TFC, FRAP and DPPH scavenging capacity were observed for the pods and the lowest for the cotyledons.

The results of this study indicate that faba beans are a good source of natural antioxidants independently to their genotype. The quantification of the antioxidant capacity according to the stage and the plant part could be suitable for applications on the food industry in relation to production and preservation of faba bean food products.

1. Introduction

A wide variety of reactive oxygen species (ROS) are produced in the course of the normal metabolism in biological systems and they have several important physiological functions, but their accumulation beyond the needs of the cell can potentially damage lipids, proteins, and nucleic acids (Cho & Kleeberger, 2007; Migliore & Coppedè, 2009). Those changes could eventually be responsible for chronic diseases including cancer (Kinnula & Crapo, 2004), cardiovascular disease (Singh & Jialal, 2006), neurodegenerative disease (Sas, Robotka, Toldi, & Vécsei, 2007) as Alzheimer, amnestic mild cognitive impairment (Butterfield et al., 2009), Parkinson's disease (Tsang & Chung, 2009) and aging (Bokov, Chaudhuri, & Richardson, 2004). In addition, lipid oxidation in food leads to a significant loss of its nutritional quality and cause the formation of toxic compounds. For this reason, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyhydro-quinone (TBHQ) are widely used in the food industry as potential inhibitors (Scherer & Teixeira-Godoy, 2009). However, their toxicological effects and the consumer preference for natural products have increased the interest for the search of natural antioxidants that might help to attenuate the oxidative processes (Viuda-Martos, Ruiz Navajas, Sánchez Zapata, Fernández-López, & Pérez-Álvarez, 2010) mainly from plant origin which have been of great concern in recent years (Liu, Qui, Ding, & Yao, 2008; Pereira de Abreu, Paseiro-Losada, Maroto, & Cruz, 2010).

The antioxidant capacity of plant foods is derived from the cumulative synergistic action of a wide variety of antioxidants such as vitamins C and E and polyphenols, mainly phenolic acids and flavonoids, carotenoids, terpenoids, Maillard compounds and trace minerals (Pérez-Jiménez et al., 2008). Polyphenols are probably the most investigated molecules of nutritional interest. Several plant polyphenols are natural antioxidants with an interesting future in various fields such as food and medicine. Because natural antioxidants have shown a reduction in oxidative stress (Osawa, 1999), some

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flavonoids have been assayed in various diseases affecting the heart, brain, and other disorders, including those leading to cancer (Núñez & Costoya, 2008; Pryor, 2000).

The leguminous family (Fabaceae) is characterized by a very wide diversity. It includes about 600 genus with 17,000 enumerated species of grain plants (Walter et al., 2001). Among them, Faba bean (*Vicia faba* L.) is the most cultivated leguminous species in the world and, in particular, in Tunisia. Its characteristics are suitable for sustainable agriculture (Nadal, Suso, & Moreno, 2003). *V. faba* is a considerable source of energy (Bond, Lawes, Hawtin, Saxena, & Stephens, 1985; Duke, 1981) with 344 Kcal/100 g. Its protein content varies from 22 to 36% (Bond et al., 1985) and it is an efficient substitute of animal protein in poor countries. The heterogeneity in the varieties of legumes and their complexity in the different phytochemical compositions lead to further research on their bioactive compounds and biological properties. Because of the mechanisms by which polyphenolic compounds present in legumes exert their biological properties have just started to be evaluated, the aim of the present study is to examine the antioxidant properties of polyphenolic compounds isolated from six parts of thirteen genotypes of faba bean.

The purpose of the present work is to evaluate and compare in vitro antioxidant activities of thirteen faba bean genotypes of four different plant parts: whole seed (WS), seed coat (SC), cotyledons (Cot) and pods (Pod) during three different stages: the maturity stage (MS) and two previous, the vegetative and the reproductive stages (VS and RS respectively), using ferric-reducing/antioxidant power (FRAP) and DPPH radical-scavenging assays. The total phenolic content (TPC) and flavonoid content (TFC) from the plant extracts were measured by colorimetric assays. From the distribution of the TPC and TFC found, it will be possible to identify the main deposits of the natural antioxidant activity in the plant.

2. Materials and methods

2.1. Chemicals

Folin-ciocalteu and gallic acid (GA) were purchased from Sigma-Aldrich (USA and China). Rutin (RUT) and catechin (CAT) were from Sigma (China and Japan). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical from Aldrich (USA). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and tert-butylhydroquinone (TBHQ) were from Fluka (Switzerland and USA). Quercitin (QR) was from Alfa Aesar (Germany). Butylated hydroxytoluene (BHT) was purchased from SAFC (Germany). All chemicals and reagent used were analytical grade.

2.2. Plant materials

Thirteen genotypes were used for this study. The strains and origins of these genotypes are resumed in Table 1. All of them were cultivated in the same open field (Chott-mariem, Tunisia) during the campaign 2006–2007 to reduce the impact of the environmental conditions.

2.3. Sample preparation

Target samples were obtained at three different stages of their life cycle. Some plants were collected in the vegetative stage, one month after their lifting. Others were collected during the reproductive stage, when all plant acrossed the flowering. And finally, at the maturity stage (MS), when the plants were completely dry. Then, they were separated in four parts: the whole seed, the seed coat, the cotyledons and the pod. The freeze-dried plant materials were ground, sieved and then stored until analyzed. A powder of each plant material (2 g) was extracted in three steps: 30 mL of ethanol 70% was added, shacked for 1 h and filtrated through a Whatman filter paper. Then 5 mL of ethanol 70% was added to the solid portion, shacked for 30 min and filtered and this process was repeated once more time. The liquid phases were mixed for consequent analysis and the solid was discarded.

Table 1
Specific information on the studied genotypes.

Genotypes	Strains	Origins
Genotype 1, G1	Major	Land race 1 (Tunisia)
Genotype 3, G3	Minor (20302)	ICARDA (Syria)
Genotype 4, G4	Minor (20104)	ICARDA (Syria)
Genotype 5, G5	Minor (20304)	ICARDA (14 crosses)
Genotype 6, G6	Minor (20301)	ICARDA (10 crosses)
Genotype 7, G7	Minor (20202)	ICARDA (14 crosses)
Genotype 8, G8	Cv diotono	Spain
Genotype 9, G9	*F. chahbi*	INRA (Tunisia)
Genotype 10, G10	Cv aquadulce	Spain
Genotype 11, G11	Major	Land race 2 (Tunisia)
Genotype 12, G12	Major	Land race 3 (Tunisia)
Genotype 13, G13	Major	Land race 4 (Tunisia)
Genotype 14, G14	Major	Land race 5 (Tunisia)

The genotypes G1, G11, G12, G13 and G14 were land races consumed by Tunisians and known in Tunisian market.
The genotypes G5, G6, G7 are improved in ICARDA.

2.4. Determination of total phenolics content

The TPC in the different stages and parts of *V. faba* was quantified using the Folin–Ciocalteu reagent according to Singleton and Rossi (1965). Even criticized for its poor specificity (Escarpa & González, 2001), this method is still mostly used because is a way to screen the total phenolics content (Liu, Qui, Ding, & Yao, 2008). Moreover it facilitates a suitable comparison of the results obtained in the present work. Briefly, 0.25 mL of the ethanolic extract of the plant was mixed with 0.25 mL of the Folin–Ciocalteu reagent (1:1) and diluted in a 10 mL volumetric flask. After 5 min, 0.5 mL of Na_2CO_3 (5%) was added. The volume was then made up to 10 mL with distilled water and mixed thoroughly. The absorbance of the reaction mixtures was measured at 720 nm against a blank after 1 h. Gallic acid (GA) was used to obtain the standard curve (0–1000 ppm). Measurements of each sample were made in duplicate and the results were expressed as milligram of gallic acid equivalent (GAE)/g of dried of plant powder.

2.5. Estimation of total flavonoids content

For the TFC the colorimetric assay reported by Zhishen, Mengchen, and Jiamming (1999) with minor modification was used. A known volume of plant extract (0.5 mL) and ethanol 60% (10 mL) were added to a 25 mL volumetric flask. At zero time, 1 mL of 5% $NaNO_2$ was added to the flask. After 5 min, 1 mL of 10% $AlCl_3$ was added and mixed. Then, after 6 min, 10 mL of 1 M NaOH was added and mixed. The solution was then made up to 25 mL with 60% ethanol and mixed. Absorbance of the mixture was measured at 510 nm versus 60% ethanol as blank. Rutin was used to run the calibration curve and the flavonoids content was expressed as milligram of rutin equivalents (RE)/g of dried plant powder.

2.6. Antioxidant capacity

2.6.1. Ferric-reducing antioxidant power assay

The antioxidant capacity of the plant extracts was performed according to the method of Benzie and Strain (1996) with some adaptations. The FRAP reagent was prepared with 10 mM of TPTZ solution in 40 mM HCl, 20 mM $FeCl_3$ solution and 0.3 M acetate buffer (pH 3.6) in proportions of 1:1:10 (v/v). Fifty microliters of each diluted ethanolic extracts was mixed with 3 mL of freshly prepared FRAP reagent and the reaction mixtures were incubated at 37 °C for 30 min. Absorbance at 593 nm was determined against distilled water blank. Aqueous solutions of ferrous sulfate (100–4000 μM) were used for calibration. Triplicate measurements were taken and the FRAP values were expressed as mmol of Fe (II)/g of dry weight of plant powder.