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Phenolics content and antioxidant and anti-inflammatory activities of legume fractions

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ABSTRACT

Two faba bean (*Vicia faba* L.) subspecies *major* and *minor* and lentil seeds grown in Algeria were separated into cotyledons and hulls. These fractions, together with their corresponding whole seeds, were extracted with two solvents, aqueous (70%) acetone and (80%) ethanol, and evaluated for antioxidant activity in relation to their phenolic contents. Acetone selectively extracted tannins from faba beans. The hulls always exhibited high antioxidant activity, measured using the reducing power (RP), antiradical activity (DPPH) or oxygen radical absorbance capacity (ORAC) assays. Aqueous ethanol (80%) extract of lentil hulls exhibited high antioxidant and anti-inflammatory activities preferentially inhibiting 15-LOX (IC_{50}, 55 μg/ml), with moderate COX-1 (IC_{50}, 66 μg/ml) and COX-2 (IC_{50}, 119 μg/ml) inhibitory effects on the COX pathway, whereas faba bean hull extracts exerted relatively mild LOX inhibitory activity.
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1. Introduction

Faba beans and lentils are pulse crops commonly grown in mixed rain fed, dry land or the agro-pastoral systems in many parts of the world, including North Africa. This region is considered the center of origin for both crops maintaining their local biodiversity. Both faba beans and lentils are still basic staple food, providing perfect complement to cereals in the diets of people in several countries. However, faba bean production has declined with increasing global pulse production (Watts, 2011). China and Canada are by far the world's largest faba bean and lentil producers, respectively accounting for 39% and 42% of global faba bean and lentil output in 2010 (FAO, 2010). Significant advances have been achieved towards harnessing the full production potential of faba bean, but value addition for new uses, such as enhancing nutritional value for new emerging functional applications and the functional foods and nutraceutical areas, remain unexplored (Salmerón, Ávila, & Torres, 2011).

Traditional faba beans have been grown worldwide and considered a major staple for the Mediterranean diet. Plant breeding has resulted in the successful development of "non-toxic" (zero tannin or tannin-free and low or reduced vicine-convicine) faba beans, primarily as a protein source for monogastric animals, particularly in Europe (Salmerón et al., 2011; Metayer, 2004). Most faba bean produced in China is processed locally, mainly for the starch widely used in primary food production, such as noodles, vermicelli, and bean sauce (Saxena, Weigand, & Li-Juan, 1993). However, the protein and hull fractions regarded as industrial by-products, have not been extensively used or exploited.

Legume seed coats, commonly referred to as hulls, are rich sources of polyphenolics and natural antioxidants (Moise, Han, Gudynaite-Savitch, Johnson, & Miki, 2005), and have been extensively investigated, both from their beneficial physiological effects in humans and deleterious effects in animal nutrition. For example, faba bean hull and its phenolics are known to suppress enzymes in the digestive tract of monogastric animals, primarily poultry and pigs, thereby reducing the bioavailability of macronutrients (Gupta, 2011). On the other hand, the hulls benefit gut microflora (Juskiewicz et al., 2006), whereas whole faba bean seeds and protein isolate reduce cholesterol and insulin levels in hypercholesterolemic patients (Weck et al., 1983).

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Faba bean and lentil polyphenolics and antioxidants have been investigated in relation to their anatomical fractions (Chaieb, González, López-Mesas, Bouslama, & Valiente, 2011; Dueñas, Sun, Hernández, Estrella, & Spranger, 2003; Vioque, Alaiz, & Girón-Calle, 2012). We have previously shown that phenolic content linearly related to tannin content is the best predictor of antioxidant activity in low-tannin faba bean genotypes (Oomah et al., 2011), and green and red lentil hulls are excellent sources of potent phenolic antioxidants (Oomah, Caspar, Malcolmson, & Bellido, 2011). However, studies are limited to traditional faba beans and lentils, particularly from the Mediterranean dry arid regions with high legume consumption. Therefore, this investigation aims to determine the phenolic compounds from traditional faba beans and lentils grown in Algeria, as well as their fractions and potential beneficial effects on antioxidant and anti-inflammatory activities. This information is critical for the development of value added products in the region, particularly as ingredient/s for the functional food and nutraceutical markets. It is important to enhance the nutritional value of traditional crops, especially those that maintain and improve soil fertility and provide regional food security.

2. Materials and methods

Legume samples consisting of faba bean (*Vicia faba* L.) subspecies *major* and *minor* were from Remila, Sidi aich and Merdi Ouamane Wilaya of Bejaia, Algeria, respectively. A blonde variety of lentil (*Lens esculenta var. Petite blonde de Dahra*) with yellow cotyledons was kindly provided by Institut Technique des Grandes Cultures (ITGC), Sétif, Algeria. Faba bean seeds were cleaned, air dried, and manually separated into hulls and cotyledons. All faba bean and whole lentil samples were initially crushed in a traditional stone mill followed by an analytic mill (IKA A11 basic; IKA Werke GmbH & Co. KG, Staufen, Germany) then sieved (Tap sieve shaker AS 200; Retsch GmbH, Haan, Germany) to pass a 500 μm screen.

Lentil hulls and cotyledons were obtained by abrasive dehulling carried out on a model 4E-230 TADD (Venables Machine Works Ltd., Saskatoon, SK, Canada) with an eight-cup cover plate described previously (Oomah, Ward, & Balasubramanian, 2010). Lentils (25 g) were placed in eight cups and dehulled for 1 min, seeds were then removed from the sample cups using the vacuum aspirating device and the dehulled seeds and hulls, were separated by air aspiration. The hulls and cotyledons were ground to a fine powder in a small coffee mill (Black & Decker Smart Grind coffee mill for 30 s). Moisture was determined by the AOAC (2005) vacuum oven method.

2.1. Acetone extraction and analysis of phenolics

Phenolics were extracted with 70% acetone as described previously (Ranalli et al., 2006). Briefly, samples (2 g) were extracted with acetone (50 ml) by magnetic stirring for 1 h at room temperature. The extract was vacuum-filtered through sintered glass (porosity 3), the residue resuspended with the same volume of solvent and the extraction repeated twice. The supernatant obtained by vacuum filtration was used for analysis of phenolics.

Total phenolics of the acetone extracts were determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). Absorbance of samples and gallic acid standards (0–0.9 mg/ml prepared in 80% ethanol) was monitored at 765 nm (SpectroScan 50 UV–visible Spectrophotometer, Sedico Ltd., Cyprus). Samples were analyzed in triplicates and results expressed in mg gallic acid equivalents (GAE)/g sample.

Tannin content of the acetone extract was determined by the bovine serum albumin BSA precipitation assay (Hagerman & Butler, 1978). Briefly, 2 ml of extract was added to 4 ml of a standard protein solution [i.e., 1 mg BSA/ml of 0.2 M acetate buffer (pH 4.2, containing 0.17 M NaCl)]. The solutions were vortexed, incubated (4 °C, 24 h), and centrifuged (1200*g*, 15 min, Centrific centrifuge model 1228, Fisher Scientific, Pittsburgh, PA). The pellet was dissolved in 4 ml of SDS-triethanolamine solution [1% (w/v) SDS and 5% (v/v) triethanolamine in deionized water]. $FeCl_3$ reagent (1 ml, 0.01 M $FeCl_3$ $6H_2O$ in 0.01 N HCl) was added, and the solution was vortexed. Absorbance was measured approximately 15 min after the addition of the iron (III) reagent at 510 nm (SpectroScan 50 UV–visible Spectrophotometer, Sedico Ltd., Cyprus) using tannic acid (0–0.12 mg/ml) as standard. The average A_{510} of triplicate samples of the SDS triethanolamine solution plus $FeCl_3$ reagent was subtracted from the A_{510} of each sample to correct for background absorbance.

The $AlCl_3$ method (Lamaison & Carnet, 1990) was used for determination of total flavonoid content of the acetone extracts. Aliquots (2 ml) of extracts were added to equal volumes of a solution of 2% $AlCl_3{\cdot}6H_2O$ (2 g/100 ml methanol). The mixture was vigorously shaken, and absorbance was monitored at 430 nm after 15 min incubation using quercetin (0–0.013 mg/ml in 80% ethanol) as standard. Flavonoid content was expressed in mg quercetin equivalents/g sample.

2.2. Ethanolic extraction and analysis of phenolics

Extraction of phenolics was performed according to the procedure described previously (Oomah, Blanchard, & Balasubramanian, 2008). Ground samples (200 mg) were extracted with aqueous ethanol 80% (v/v) (8 ml) by constant magnetic stirring (RT15 power S1, IKA Werbe GmbH & Co. KG, Staufen, Germany) for 2 h at room temperature. After centrifugation (1100*g*, 10 min; IEC HN-SII Centrifuge, International Equipment Company, Needham Heights, MA), the recovered supernatant was stored at −20 °C in the dark until analysis. Phenolic content of ethanol extracts was determined according to the previously described procedure (Oomah, Corbé, & Balasubramanian, 2010). Briefly, the method consisted of adding 100 μl of sample extract with 150 μl of a solution of 2% HCl in 80% ethanol in a 96-well ultra violet flat-bottom plate (Greiner Bio-One Inc., Longwood, FL). The absorbance of the solution was monitored at 280, 320, 360, and 520 nm after mixing for 3.5 min with a spectrophotometer (Spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA) using (+)-catechin (0–241 μg/ml), caffeic acid (0–36 μg/ml), quercetin (0–31.6 μg/ml), and cyanidin-3-glucoside (0–80 μg/ml) as standards for total phenolics, tartaric esters, flavonols and anthocyanins, respectively. Standards were prepared in aqueous ethanol 80% (v/v). The absorbance was also monitored at 710 nm for turbidity correction and the results were expressed in mg (+)-catechin, caffeic acid, quercetin or μg cyanidin-3-glucoside equivalents/g sample.

Tannin content of ethanol extract was determined as described previously (Ross, Zhang, & Arntfield, 2010) using insoluble polyvinylpolypyrrolidone (PVPP, Sigma–Aldrich Canada Ltd., Oakville, ON, Canada). Briefly, 80% ethanol extract (1 ml) was added to 50 mg of PVPP, stirred for 20 min at 22 °C and then microfuged (12000 rpm, 5 min, Mini spin plus, Eppendorf, Hamburg, Germany) prior to determination of total phenolics. Tannin content was calculated as the difference between total phenolics and non-tannin phenolics.

2.3. HPLC analysis of phenolic acids

Chemicals (methanol, acetic acid) used for high-performance liquid chromatography (HPLC) were of chromatographic grade (Sigma–Aldrich Canada Ltd., Oakville, ON, Canada). Analysis of phenolic acids in the ethanol extracts of lentil hulls was carried

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