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Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (*Vicia faba* L.) differing in seed coat colours

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ABSTRACT

The Australian grown faba beans of different seed coat colours were either soaked, boiled or autoclaved, and analysed for phenolic contents and antioxidant activity using an array of reagent-based assays. Soaking, boiling and autoclaving were shown to lower the level of active compounds in faba beans. A significant amount of active compounds was leached to the soaking and cooking medium. Boiling was a better method in retaining active compounds in beans than autoclaving. The boiled beans had more active compounds than those of resulting cooking broths, which was the opposite observation when autoclaving. The buff-genotypes had a similar level of active compounds to red- and green-genotypes. The high performance liquid chromatography-post column derivatisation (HPLC-PCD) system detected a dense collection of high antioxidant HPLC peaks ('humps') in extracts of raw, soaked and boiled beans. The present findings encouraged consumption of faba beans together with cooking broth for the maximum potential health benefits.

1. Introduction

Many staple dishes prepared from pulses are cooked in a variety of ways. For example, faba beans (broad beans) are served as Ful Medames in Egypt and the fried-faba beans are served as snack foods in Asia; lentils and/or chickpeas are stewed to form a thick paste, dhal, which is served with rice or roti in India and other Southern Asia countries; adzuki beans are regularly utilised in patisserie desserts and bakery fillings in Japan; black beans or other types of beans are served as Feijoada in some African countries. Pulses, including cowpeas (black-eyed peas), black beans and mung beans, are used as ingredients for soups and desserts in Chinese and South East Asian cuisines.

Epidemiological studies have shown that intake of whole-grain cereals and legumes are associated with lowered risk of chronic diseases such as cardiovascular diseases, type-2 diabetes, and cancers (Bazzano, Thompson, Tees, Nguyen, & Winham, 2011; McKeown, Meigs, Liu, Wilson, & Jacques, 2002; Mellen, Walsh, & Herrington, 2008; Nagura et al., 2009; Schatzkin, Park, Leitzmann, Hollenbeck, & Cross, 2008). The minor-constituents, including phenolic compounds in whole-grain foods, are thought to play important roles in disease prevention. On the other hand, phenolic compounds are also known to contribute to sensory traits, appearance and functionalities of food products particularly when interacting with proteins (O'Connell & Fox, 2001; Siebert, Troukhanova, & Lynn, 1996).

Many types of pulses, including faba beans (*Vicia faba* L.) have been reported to contain high phenolic contents and antioxidant capacities (Amarowicz, Karamac, Kmita-Glazewska, Troszynaska, & Kozlowska, 1996; Siah, Konczak, Agboola, Wood, & Blanchard, 2012). The phenolic contents and antioxidant capacities of beans with different seed coat colours vary (Siah et al., 2012). Since pulses are not commonly eaten raw, a variety of thermal processing methods are applied to pulses to achieve desirable sensory and, sometimes, nutritional properties. Soaking pulses prior to thermal processing is a common practice to shorten the cooking time.

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Different processing methods, including soaking, boiling, steaming, roasting and pressure cooking, have a significant effect on phenolic contents and antioxidant levels of various types of pulses (Baoteng, Verghese, Walker, & Ogutu, 2008; Xu & Chang, 2008). The phenolic content and antioxidant activities of faba beans are also affected by cooking (Amarowicz, Troszynska, Barylko-Pikielna, & Shahidi, 2004; Chaieb, Gonzalez, Lopez-Mesas, Bouslama, & Valiente, 2011). However, studies of phenolic content and antioxidant activity of dry faba beans as affected by thermal processes are limited, particularly with regards to faba beans grown in Australia, which contribute to about 20% of the world export market (FAOSTAT, 2010). Furthermore, previous reports have not considered cooking broth, but focused on processed beans only (Khalil & Mansour, 1995; Vidal-Valverde et al., 1998), therefore any leaching of phenolic compounds into cooking broths has not been quantified.

This study aimed to determine the effect of soaking, boiling and autoclaving (pressure cooking) on the phenolic contents and antioxidant activities of Australian grown faba bean genotypes differing in seed coat colour. The phenolic compounds extracted from faba beans and cooking broths were also characterised.

2. Materials and methods

2.1. Faba bean samples

Five faba bean genotypes with different seed coat colours including red-coloured variety *Rossa*, green-coloured variety *Icarus*, buff-coloured varieties *Doza* and *Nura, and white-coloured low tannin breeding line TF(Ic*As)*483/13* were selected for this study. All genotypes were grown to maturity at Wagga Wagga Agricultural Institute, Australia in 2008 and mechanically harvested. Bean samples were cleaned by removing foreign materials, loosely packed and stored in the dark at room temperature for five months to naturally dry, followed by storing at −18 °C.

2.2. Processing

2.2.1. Soaking

Raw faba bean seeds (10 g) were added to 50 ml of distilled water, allowed to soak for 12 h overnight at room temperature (Nasar-Abbas et al., 2008). After soaking, the water was drained and the soaked beans were blotted dry using paper towels. Soaked beans were added to 100 ml of aqueous acetone (acetone:water, 70:30, v/v) and the extraction of the phenolic compounds was performed (refer to Section 2.3).

2.2.2. Boiling

Soaked beans (from 10 g of unprocessed faba beans prepared as described in Section 2.2.1) were added to 100 ml of boiling distilled water and allowed to boil at atmospheric pressure for 40 min. The cooking broth was drained, freeze dried, and kept at −18 °C for analysis without subsequent solvent extraction. The boiled beans were centrifuged at 2000*g* for 5 min at 4 °C to remove excess water. A solution of aqueous acetone was added and the phenolics were extracted (refer to Section 2.3). Five faba bean genotypes: *TF(Ic*As)*483/13*, *Doza*, *Nura*, *Icarus* and *Rossa*, were selected to undergo soaking and boiling treatments.

2.2.3. Autoclaving

Raw faba bean seeds (10 g) were added to 50 ml of low calcium content distilled water (80 mg of calcium chloride/L) and allowed to soak for 12 h at room temperature (Revilla & Vivar-Quintana, 2008). After draining the soaking solution, a solution of 2% sodium chloride (100 ml) was added to the soaked beans and autoclaved at 115 °C for 20 min. The cooking broth was drained, freeze-dried and kept at −18 °C for analysis without subsequent extraction using solvents. The autoclaved beans were centrifuged at 2000*g* for 5 min at 4 °C to remove excess water. A solution of aqueous acetone was used for phenolic extraction (refer to Section 2.3). The soaked beans of three faba bean genotypes, *TF(Ic*As)*483/13*, *Nura* and *Rossa*, were chosen to undergo the autoclaving processes.

2.3. Preparation of crude phenolic extracts

Phenolic compounds from soaked, boiled and autoclaved beans were extracted using 70% acetone (v/v) followed by freeze drying as previously described (Siah et al., 2012). The extraction yields (g/gDW) of acetone extract from the unprocessed or processed bean portions were based on the dry weight of beans or bean powder. The resulted cooking broths were sifted to remove the coarse particles and the collected clear broths were freeze-dried. The extraction yields (g/gDW) of freeze dried materials from the clear broths were calculated based on the dry bean weight. These freeze-dried extracts and the freeze dried cooking broths (from Sections 2.2.2 and 2.2.3) were dissolved in deionized water and filtered with a 0.4 μm filter (Millipore) before performing further analysis.

2.4. Chemical assays

2.4.1. Total phenolic content, total flavonoid content and antioxidant capacity assays

The total phenolic content (TPC), total flavonoid content (TFC), diphenylpicrylhydrazyl (DPPH) radical-scavenging capacity, Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC) assays were performed as previously described (Siah et al., 2012). The proanthocyanidin content (TPro) assay was carried out according to the hydrochloric acidified 4-dimethylaminocinnamaldehyde (DMAC–HCl) protocol as described by Li, Tanner, and Larkin (1996), with a reduced volume to fit in a 96-well microplate. The oxygen radical absorbance capacity (ORAC) assay was performed as outlined by Prior et al. (2003) using a FLUO star Omega UV–Vis microplate reader (BMG Lab technologies, Offenburg, Germany). The ferric reducing antioxidant power (FRAP) assay was used to measure the ferric reducing ability of faba bean extracts (Konczak, Zabaras, Dunstan, & Aguas, 2010).

2.5. On-line post-column derivatisation (PCD) with high performance liquid chromatography (PCD-HPLC)

Phenolic compound profiling and antioxidant activity analysis was carried out on-line using $ABTS^{\cdot+}$ cation radicals as previously described (Ee, Agboola, Rehman, & Zhao, 2011; Siah et al., 2012). The HPLC system (ProStar model 410) consisted of a Phenomenex Luna 5U C18 column (100 A pore size; 150 × 3 mm), preceded by a guard column (Phenomenex, 4 × 3 mm), a Varian 240I pump and a Varian 335 PDA Detector. The mobile phase A was water–acetic acid (99:1; v/v) and phase B was methanol–acetonitrile (50:50; v/v). An aliquot (8 μl) of extract sample (50 mg/ml) dissolved in solvent A was injected and eluted in a gradient of 0–48% phase B for 40 min at a flow-rate of 0.4 ml/min. UV spectra were recorded at 280 nm. Post column antioxidant activity on-line was determined on the HPLC eluent from the system which arrived at a "T" piece and reacted with $ABTS^{\cdot+}$ that was added at a flow rate of 0.4 ml/min. The absorbance of the reaction products was measured by a UV–Vis detector at 414 nm.

2.6. Statistical analysis

Two independent acetone extractions were performed on the raw beans and three on the soaked, boiled and autoclaved beans.