Journal of Functional Foods xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Antioxidant activity of broad bean seed extract and its phenolic composition

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ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form 27 March 2017
Accepted 1 April 2017
Available online xxxx

Keywords:
Broad bean
Antioxidant activity
Phenolic compounds

ABSTRACT

The crude acetonic extract of broad bean seeds was separated into fraction I (low-molecular-weight compounds) and fraction II (tannin fraction) using Sephadex LH-20 column chromatography with ethanol and acetone/water (1:1, v/v) as the mobile phases. Phenolics present in the crude extract and fractions thereof showed antioxidant and radical-scavenging properties determined using several methods (emulsion system, ABTS, DPPH, and reducing power assays). Fraction II exhibited higher antioxidant efficacy than those of the crude extract and fraction I. In the crude extract, 14 compounds, namely phenolic acids (*p*-coumaric, ferulic), catechins (epicatechin, epicatechin glucoside, epicatechin gallate), procyanidin gallate, prodelphidin dimer, gallate procyanidin dimer, and digallate procyanidin dimer, were identified by HPLC-DAD-MS. Catechin gallate, digallate procyanidin dimer, and gallate procyanidin dimer were the major phenolics present in the extract.

Contents

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http://dx.doi.org/10.1016/j.jff.2017.04.002
1756-4646/

1. Introduction

Phenolic compounds occur in different anatomical parts of plants. They can be classified into several classes: phenolic acids (hydroxybenzoic and hydroxycinnamic acids and their derivatives), flavonoids, lignans, stilbenes, and condensed as well as hydrolyzable tannins. Phenolic compounds of plant origin can inhibit or delay the oxidation in food and in the body due to their antioxidant potential. Reactive oxygen and nitrogen species (RONS) that affect lipids, proteins and DNA can be scavenged by natural antioxidants present in the human diet. (Halliwell, Gutteridge, & Cross, 1992; Willett, 1994). Epidemiological studies have demonstrated the protective effect of consumption of phenolic-rich foods against several chronic diseases (Kris-Etherton et al., 2002; Kushi, Meyer, & Jacobs, 1990).

In the last couple of decades, there has been a growing interest in finding natural sources of antioxidants in plant foods, including pulses and their potential health benefits associated with protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative ailment. Pulses are important food sources and play a significant role in traditional diets in many regions of the world (Flight & Clifton, 2006; Vaz Patto et al., 2015). Several research findings have indicated that they serve as a rich source of natural antioxidants for health promotion and reduction of disease risk (Heimler, Vignolini, Dini, & Romani, 2005; Madhujith & Shahidi, 2005; Shahidi & Ambigaipalan, 2015; Xu & Chang, 2007). Potential health benefits of pulses in cardio-protection (Bouchenak & Lamri-Senhadji, 2013; Kushi et al., 1990) as well as prostate and breast cancer prevention (Kolonel et al., 2000; Peeters, Boker, van der Schouw, & Grobbee, 2003) have previously been reported. These antiproliferative and antioxidant effects of legumes have been associated with the presence of different classes of phenolic compounds (Adebamowo et al., 2005; Aparicio-Fernandez et al., 2008).

Broad bean and faba bean (*Vicia faba*) are the species of Fabaceace family and belong to the same genus but differ in their growth requirements, markets and end-uses. In parts of the English-speaking word (United Kingdom, Australia, New Zealend), the name "broad bean" is used for the large-seeded cultivars grown for human food, while "faba bean" refers to cultivars with smaller, harder seeds used for animal feed. *Vicia faba* is also knows as "fava bean", "field bean", "hors bean", "Windsor bean".

Broad bean seeds are a rich source of bioactive compounds such as phenolic or polyphenolic compounds, including tocopherols, as well as triterpenic acids (Abu-Reidah, del Mar Contreras, Arráez-Román, Fernández-Gutiéraz, & Segura-Carretero, 2014; Klogeropoulse et al., 2010; Łabuda & Buczkowska, 2014). Several research reports have confirmed the antioxidant potential of broad bean seeds using different chemical methods (Amarowicz, Karamać, Kmita-Głażewska, Troszyńska, & Kozłowska, 1996; Amarowicz & Raab, 1997; Yao, Cheng, Wang, Wang, & Ren, 2010).

The extracts of phenolic compounds of broad bean have been examined and shown to have inhibitory effects towards lipases, lipoxygenases, and α-amylase (Al-Obaidy & Siddiqi, 1981; Borowska, Giczewska, & Zadernowski, 2003; Zadernowski, Borowska, Naczk, & Nowak-Polakowska, 2001). Boudjou, Oomah, Zaidi, and Hosseinian (2013) reported the inhibition of lipoxygenase by broad bean hull extract expressed as IC_{50} of 192 μg/ml.

Despite many reports on health benefits of a variety of seeds, including pulses, there is still a gap in the existing knowledge on certain details that require additional research in order to adequately reveal the composition of their extracts and to identify the antioxidant compounds in them for better utilization of pulse and their extracts as natural antioxidants and food supplement. In our opinion very important is the contribution of condensed tannins in the antioxidant potential of the crude extract. The phenolic composition of broad bean extract is not adequately described in literature. Therefore the aim of the present study was to determine the polyphenolic profiles and antioxidant properties of broad bean extract and its low-molecular-weight as well as tannin fractions.

2. Materials and methods

2.1. Chemicals

Hexanes, acetone, methanol, acetonitrile, ethanol, potassium ferricyanide, and trichloroacetic acid (TCA) were acquired from the P.O.Ch. Company (Gliwice, Poland). Sephadex LH-20, linoleic acid, β-carotene, polyoxyethylenesorbitan monopalmitate (Tween 40), polyoxyethylenesorbitan monopalmitate (Tween 40), butylated hydroxyanisole (BHA), 6-hydroxy-2,5,7,8,-tetram-ethylchroman-2-carboxylic acid (trolox), Folin & Ciocalteu's phenol reagent, vanillin, and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma (Poznań, Poland). *p*-Hydroxybenzoic acid, *trans*-*p*-coumaric acid, *trans*-ferulic acid (+)-catechin, (−)-epicatechin, dihydroquercetin, and tryptophan were purchased from Extrasynthese (Genay Cedex, France).

2.2. Plant materials

Authenticated broad bean (*Vicia faba* major) seeds of *Albus* cultivar were obtained from the Plant Breeding Station in Olsztyn, Poland.

2.3. Extraction

Lipids were removed from ground broad beans using hexanes and in a Soxhlet apparatus. Phenolic compounds were subsequently removed using 80% (v/v) acetone at a solids-to-solvent ratio of 1:10 (w/v) (Amarowicz et al., 1996). Extraction was carried at 50 °C for 30 min in flasks placed in a shaking water bath (Elpan 357, Wrocław, Poland). Extraction was repeated twice and the solvent from combined supernatants was removed under vacuum at 40 °C using a Büchi rotary evaporator. The sample was then freeze-dried.

2.4. Column chromatography

The chromatographic method of Strumeyer and Malin (1975) was employed for separation of the low-molecular-weight phenolics and tannin fractions from the crude extract. A glass chromatographic column (5 × 50 cm) packed with Sephadex LH-20 was equilibrated with 95% (v/v) ethanol and to which the crude extract was subsequently applied. Fraction 1 composed of the low-molecular-weight phenolic compounds was obtained by using approximately 1.2 L of ethanol as the mobile phase. Fraction II (tannins) was eluted 600 mL of 50% (v/v) acetone. Ethanol end acetone were evaporated and any remaining water from tannin fraction II was freeze-dried.

2.5. Determination of total phenolic compounds

The method described by Naczk and Shahidi (1989) was used for determination of total phenolic compounds in the crude extract and fractions I and II. The results were expressed as (+)-catechin equivalents per g of the extract or fraction thereof.

Please cite this article in press as: Amarowicz, R., & Shahidi, F. Antioxidant activity of broad bean seed extract and its phenolic composition. *Journal of Functional Foods* (2017), http://dx.doi.org/10.1016/j.jff.2017.04.002