Journal of Functional Foods 24 (2016) 359–372

Available online at www.sciencedirect.com

ScienceDirect

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

Antioxidant property and their free, soluble conjugate and insoluble-bound phenolic contents in selected beans

Yu-Kun Wang [a], Xin Zhang [b], Guan-Lin Chen [c,*], Jing Yu [a], Lu-Qi Yang [a], Yong-Qing Gao [a,**]

[a] School of Food Science, Guangdong Pharmaceutical University, Zhongshan 528458, China
[b] Zhejiang GIIAN Testing Technology Institute, Zhejiang 310021, China
[c] Foshan Institute of Occupational Disease Prevention and Control, Foshan 528000, China

ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form 21 April 2016
Accepted 25 April 2016
Available online 4 May 2016

Keywords:
Antioxidant capacity
Alkaline hydrolysis
Phenolic content
Phaseolus vulgaris L.

ABSTRACT

To explore the potential of common beans as natural sources of bioactive compounds, the antioxidant properties and total phenolic content (free, soluble conjugate, insoluble-bound fraction) of 14 beans from China were evaluated systematically. The results showed that beans had diverse antioxidant capacities and the variation was very large. Black bean, flower waist bean, pearl bean and spring bay bean showed the strongest antioxidant activities among the 14 tested samples based on a combinative consideration of the results obtained by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) assay, ferric reducing antioxidant power (FRAP) assay, and trolox equivalent antioxidant capacity (TEAC) assay. Furthermore, several phenolic compounds were detected; gallic acid and protocatechuic acid were widely found in these beans. The results implied that these beans were important natural sources for preventing oxidative stress diseases.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes, consumed worldwide as pods of green beans or seeds of dry beans (Takeoka et al., 1997; Tsuda, Osawa, Ohshima, & Kawakishi, 1994). The common beans are rich and inexpensive sources of proteins, carbohydrates, dietary fibres, minerals and vitamins to millions of people in developed and developing countries (Rehman, Salariya, & Zafar, 2001). In the last decade, several studies have been focused on characterizing the phytochemicals and health benefits of common beans, and results indicated that common beans and soybeans may serve as excellent dietary sources of natural antioxidants for disease prevention and health promotion (Madhujth, Naczk, & Shahidi, 2004; Oomah, Cardador-Martínez, & Loarca-Piña, 2005; Xu & Chang, 2007). The associations between the consumption of beans and reduced risk of cardiovascular disease,

* *Corresponding author.* Foshan Institute of Occupational Disease Prevention and Control, Foshan 528000, China. Tel.: +86 57 82902590; fax: +86 57 83375959.
E-mail address: lin728335493@163.com (G.-L. Chen).
** *Corresponding author.* School of Food Science, Guangdong Pharmaceutical University, Zhongshan 528458, China. Tel.: +86 60 88207955; fax: +86 60 88207956.
E-mail address: yongqingg@163.com (Y.-Q. Gao).

Abbreviations: FRAP, ferric reducing antioxidant power; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2-azinobs-(3-ethylbenzothiazoline -6-sulfonic acid); UPLC, Ultra Performance Liquid Chromatography

http://dx.doi.org/10.1016/j.jff.2016.04.026
1756-4646/

diabetes mellitus, obesity, cancer and diseases of digestive tract also have been demonstrated (Bazzano, He, Ogden, & Et, 2001; de Mejıa, Castaño-Tostado, & Loarca-Piña, 1999; Deschasaux et al., 2014; Ha et al., 2014; Thompson et al., 2012). These potential health benefits of beans have been attributed to the presence of secondary metabolites such as phenolic compounds that possess antioxidant properties (Azevedo et al., 2003; Cardador-Martínez, Loarca-Piña, & Oomah, 2002; Lazzé et al., 2003). Phenolic compounds constitute one of the most numerous and ubiquitously distributed groups of plant secondary metabolites and are responsible for various beneficial effects in a multitude of diseases (Harborne & Williams, 2000; Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005). They exist in free, soluble conjugated and insoluble bound forms (Jung, Jeon, & Bock, 2002; Krygier, Sosulski, & Hogge, 1982). Soluble conjugate phenolics are also known as esterified phenolics (Alshikh, de Camargo, & Shahidi, 2015). It has been extensively reported that phenolic compounds have a high antioxidant activity (Chandrasekara & Shahidi, 2010, 2011a; Chen et al., 2014; El-Seedi et al., 2012; John & Shahidi, 2010; Liyana-Pathirana & Shahidi, 2005, 2006a, 2006b; Liyanapathirana & Shahidi, 2004). Although the nutritional and chemical components and their bioactivities contained in common beans have been reported (Guajardo-Flores, Serna-Saldívar, & Gutiérrez-Uribe, 2013; Lee, Hung, & Chou, 2007; Moreno-Jiménez et al., 2015; Rocha-Guzmán, González-Laredo, Ibarra-Pérez, Nava-Berúmen, & Gallegos-Infante, 2007; Valdés, Coelho, Michelluti, & Tramonte, 2011; Xu & Chang, 2011), there is still limited literature on the profile (free, soluble conjugate, insoluble-bound) of phenolic compounds and total antioxidant activity of common beans. The objective of the present study was to investigate the phytochemical profiles that exist in the free, soluble conjugate, and insoluble-bound forms, as well as their antioxidant activity in selected beans.

2. Materials and methods

2.1. Materials

Acetonitrile was HPLC grade and purchased from Merck (Darmstadt, Germany). Formic acid was HPLC grade and purchased from CNW Technologies GmbH. Gallic acid, (+)-catechin, epicatechin, protocatechuic acid, caffeic acid, vanillic acid, ferulic acid, *p*-coumaric acid, quercetin, isoquercitrin, rutin, quercitrin and syringic acid were bought from Yanshengshiye Co. Ltd. (Shanghai, China) and National Institutes for Food and Drug Control of China. The 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ), 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) were purchased from Aladdin Industrial Inc. The 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and the Folin–Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in the experiments were of analytical grade, and deionized water was used.

2.2. Sample preparation

Fourteen beans were studied, namely, black bean, chickpea, cow gram, flower waist bean, hyacinth bean, kidney bean, mung bean, pearl bean, *Phaseolus calcaratus*, red bean, red kidney bean, semen dolichoris, soy bean, and spring bay bean. The beans were collected from the local markets in Fo-shan, China. The dry beans were ground to a fine powder with a special grinder for herbal medicine and passed through a 20-mesh sieve. The particle size was 0.8 mms.

2.3. Separation of phenolic fractions using alkaline hydrolysis

The free, soluble conjugate and insoluble-bound phenolic acids from beans were prepared using the procedure explained in the literature (Ghiselli, Nardini, Baldi, & Scaccini, 1998; Krygier et al., 1982; Madhujith & Shahidi, 2009) with a slight modification. Twenty millilitres of 40% ethanol was added to 1.0 g of the powder, and then samples were ultrasound for 10 min to extract phenolic content at room temperature (23–25 °C). The mixture was centrifuged at 4000*g* for 5 min to obtain free phenolic acids. The residue was extracted with the same solvent twice, and the supernatants were combined. The supernatants were evaporated at 50 °C in vacuo, followed by extracting 3 times with ethyl acetate at 1:1 (v/v) solvent to supernatant ratio. The combined extracts were evaporated to dryness in vacuo at 50 °C, and subsequently dissolved in 50% methanol to obtain free phenolic acid fraction. The supernatant containing soluble conjugates (water phase) was subsequently hydrolysed with 30 mL of 4 M NaOH under N_2 for 4 h at room temperature. The resultant hydrolysate was acidified to pH 2 using 6 M HCl followed by extraction with ethyl acetate 3 times. The ether extracts were combined and evaporated to dryness in vacuo at 50 °C and subsequently dissolved in 50% methanol to obtain soluble conjugate fraction. The solid residues were treated with 20 mL of 4 M NaOH and hydrolysed for 4 h at room temperature under a stream of N_2 and acidified to pH 2 with 6 M HCl and centrifuged at 4000*g* for 5 min. The supernatant was extracted with ethyl acetate 3 times. The combined extracts were evaporated to dryness at 50 °C in vacuo and subsequently dissolved in 50% methanol to obtain insoluble-bound phenolics.

2.4. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure described in the literature (Benzie & Strain, 1996). Briefly, the FRAP reagent was prepared from 20 mmol/L iron(III) chloride solution, 10 mmol/L TPTZ solution in 40 mmol/L HCl and 300 mmol/L sodium acetate buffer (pH 3.6) in a volume ratio of 1:1:10, respectively. FRAP reagent should be prepared fresh daily and warmed in a water bath at 37 °C before use. Then 1 mL of the diluted sample was added to 3 mL of the FRAP solution and the absorbance was determined at 593 nm after 2 h using a Rui Li U9600 UV–vis spectrophotometer (Beijing, China) (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). Trolox was used as a reference standard, and the results were expressed as μmol Trolox/g dry weight of beans.

2.5. DPPH radical scavenging activity

Antioxidant activities of the samples were analysed by investigating their abilities to scavenge the DPPH$^{\bullet}$ (Cai, Sun, & Corke,