Food Research International 51 (2013) 518–525

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals

Weiwei Guo, Trust Beta *

Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

ARTICLE INFO

Article history:
Received 29 August 2012
Accepted 2 January 2013

Keywords:
Whole-grain cereals
Insoluble dietary fibre
Soluble dietary fibre
Alkaline extracts
Antioxidant activity

ABSTRACT

The consumption of whole-grain cereals (WG) has been revealed to be associated with the reduced risk of developing chronic diseases. Cereal dietary fibre (DF), as an abundant source of plant cell wall bound hydroxycinnamic acids, can be considered as an important contributor to the health benefit of WG consumption. In the present study, major phenolic acids in WG and DF alkaline extracts were analysed by reversed phase high performance liquid chromatography (HPLC) coupled with photodiode array detection (PDA) and quadrupole-time of flight mass spectrometry (Q-TOF MS). Eight monomeric phenolic acids (protocatechuic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic, and *iso*-ferulic acid) and four dimeric ferulic acids (diFA) (8-5′, 5-5′, 8-*O*-4′, and 8-5′-benzofuran diFA) were identified and quantified in cereal samples. Ferulic acid was detected as the predominant monomeric phenolic acid and 8-*O*-4′ diFA was the major dimeric ferulic acid. The results of total phenolic content (TPC) measured by Folin–Ciocalteau assay and antioxidant activity (AOA) evaluated by DPPH radical scavenging assay indicated that insoluble dietary fibre (IDF) alkaline extracts had significantly ($p<0.05$) higher levels of TPC and AOA than WG and soluble dietary fibre (SDF) extracts for the same sample. Corn IDF with its higher levels of TPC, ferulic acid content, and AOA can be regarded as a potential antioxidant value-added functional food ingredient.

1. Introduction

Cereal grains are major food sources worldwide. The inverse correlation between the intake of whole-grain cereals (WG) and the risk of chronic disease, such as cardiovascular disease, type II diabetes, obesity, and cancer, has been revealed (Okarter & Liu, 2010). These health benefits are in part attributed to the antioxidant capacity of phenolic compounds in cereals (Liu, 2007). The predominant group of phenolic antioxidants in cereal grains is phenolic acids, which can be further classified into hydroxybenzoic acids and hydroxycinnamic acids. Hydroxycinnamic acid derivatives, particularly ferulic acid, are the most abundant type of phenolic acids in cereal grains and found esterified or etherified to cereal cell wall components. As the major constituent of plant cell walls, dietary fibre (DF) is regarded as an abundant source of phenolic acids. Ferulic acid, *p*-coumaric acid, sinapic acid, and some phenolic acid dehydrodimers were detected in DF fractions from a wide variety of cereal grains (Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001; Bunzel et al., 2003; Dobberstein & Bunzel, 2010; Lam, Kadoya, & Iiyama, 2001; Ralph et al., 2004). The DF polysaccharides linked phenolic acids can be released by bacterial enzymes in the large intestine (Buchanan, Wallace, Fry, & Eastwood, 1996; Kroon, Faulds, Ryden, Robertson, & Williamson, 1997; Vitaglione, Napolitano, & Fogliano, 2008). The free phenolic components can function as antioxidants and provide an important health protection in the human body (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011; Poquet, Clifford, & Williamson, 2008; Wang, Sun, Cao, & Wang, 2011). Therefore, to reveal the antioxidant potential of DF linked phenolic compounds of cereal grains, the objectives of the present study were to confirm the phenolic acid composition of DF alkaline extracts obtained from barley, wheat, corn, rice and oats and to evaluate the antioxidant capacity of DF alkaline extracts based on chemical assays.

2. Materials and methods

2.1. Samples and chemicals

Seven different species of WG include barley (Lot no. 100526A), purple barley (Lot no. 9999001117), wheat (MSU D8006 soft white wheat), purple wheat, yellow corn (US P1395 XR), red rice (Jatiluwih red rice, Bali, Indonesia), and oats (Lot no. 10702tN11010AA) were used in the present study.

Heat-stable α-amylase Termamyl 120L, protease Alcalase 2.4L, and amyloglucosidase AMG 300L were used in DF preparation (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). Phenolic acid standards (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringe, *p*-coumaric, ferulic, sinapic, *iso*-ferulic, *o*-coumaric, *trans*-cinnamic acid) were used for phenolic acid identification and quantification

* Corresponding author. Tel.: +1 204 474 8214; fax: +1 204 474 7630.
E-mail address: Trust.Beta@ad.umanitoba.ca (T. Beta).

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http://dx.doi.org/10.1016/j.foodres.2013.01.008

(Sigma-Aldrich Chemical Co., St. Louis, MO, USA). Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox) were applied for antioxidant potential evaluation (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). Ethanol, acetone, and ethyl acetate were used for sample preparation and deionized distilled water, HPLC grade methanol and acetic acid were used in HPLC and HPLC–MS/MS analysis.

2.2. Dietary fibre (DF) preparation

WG were ground to pass through a 0.5 mm sieve screen for DF preparation. Insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) from WG were prepared based on AOAC Official Method 991.43 and Bunzel et al. (2001) with modifications. Briefly, 5 g of milled sample in duplicate was subjected to sequential enzymatic digestion by heat-stable α-amylase (250 μL, boiling water bath for 30 min), alcalase protease (50 mg/mL, 500 μL, pH 7.5, 60 °C water bath for 30 min), and amyloglucosidase (1500 μL, pH 4.5, 60 °C water bath for 30 min) to remove starch and protein. After centrifugation (10,000 rpm, SS-34 Rotors, RC5C Sorvall Instruments, Thermo Scientific, Asheville, NC, USA), the residue that had been washed with hot distilled water, ethanol (95%), and acetone (95%) was vacuum filtered and referred to as IDF. The supernatant combined with the washings of the residue was precipitated in ethanol (80%, preheated to 60 °C, 4 volumes) overnight and referred to as SDF. Both the IDF and SDF were placed in a fume hood to remove organic solvent and then dried at 35–40 °C overnight in a vacuum oven (Model 5831, Napco Scientific Company, Tualatin, OR, USA). The dried fibre fractions were ground into fine powder by using a mortar and pestle and then passed through a 0.5 mm sieve. The procedures described were repeated several times to get sufficient IDF and SDF for further analysis.

2.3. Phenolic acid extraction

Alkaline hydrolysis and liquid–liquid partitioning steps were applied to release ester linked phenolics from WG and their fibre fractions (Bunzel et al., 2001, 2003; Grabber, Hatfield, Ralph, Zon, & Amrhein, 1995). Specifically, WG (100 mg), IDF (100 mg), and SDF (50 mg) were placed in dark-coloured screw-cap bottles with degassed NaOH (4 M, 5 ml). The head-space of the bottles was flushed with a stream of N_2 to remove the air. The bottles were shaken slowly on a rotary shaker (Fermentation Design Inc., Allentown, PA) for 4 h at room temperature and in the absence of light. Samples were then acidified with HCl (6 M) to pH 1.5–2 and extracted with ethyl acetate (twice the volume, three times). After centrifuging (Thermo Scientific IEC CL31 Multispeed Centrifuge, Thermo Fisher Scientific Inc., USA) at 4000 rpm for 20 min, the supernatants obtained from each time were combined and dried to consistent weight by using a rotary evaporator (IKA RV10, IKA® Works Inc., North Carolina, USA). The dried alkaline extracts were re-dissolved in 1 mL of MeOH/H_2O (50/50, v/v) for the determination of total phenolic content and DPPH radical scavenging activity. Extracts were filtered with a 0.45 μm PTFE filter before HPLC and HPLC–MS/MS analysis.

2.4. HPLC and HPLC–MS/MS analysis

The separation of major phenolic acids in WG and DF alkaline extracts was carried out on a reversed phase high performance liquid chromatography (HPLC) (Waters 2695) coupled with a photodiode array detector (PDA) (Waters 996), an autosampler (Waters 717 plus), and a quadrupole-time of flight mass spectrometry (Q-TOF MS) (Micromass, Waters Corp., Milford, MA). The HPLC column was a 25 cm × 4.6 mm, 5 μm RP C18 column (Shim-pack HRC-ODS, Shimadzu Corp., Tokyo, Japan). The mobile phase consisted of A (0.1% acetic acid in water) and B (0.1% acetic acid in methanol). A 70 min-linear gradient established in our laboratory was applied as follows: 0–11 min, 9–14% B; 11–14 min, 14–15% B; 14–17 min, 15–15% B; 17–24 min, 15–16.5% B; 24–28 min, 16.5–19% B; 28–30 min, 19–25% B; 30–36 min, 25–26% B; 36–38 min, 26–28% B; 38–41 min, 28–35% B; 41–46 min, 35–40% B; 46–48 min, 40–48% B; 48–53 min, 48–53% B; 53–70 min, 53–70% B. The injection volume of sample was 10 μL and the flow rate was 0.9 mL/min. The monitor wavelength was set at 280 nm. The mass spectra (MS) were recorded in negative ion mode by using the following conditions: capillary voltage 1500 V, cone voltage 30 V, desolvation gas (N_2) flow rate 900 L/h, cone gas (He) flow rate 50 L/h, desolvation gas temperature 300 °C, ion source temperature 150 °C, and mass range 100–1000 amu. The tandem MS spectra (MS/MS) were acquired by using the collision energy of 10 V for monomeric phenolic acids and 30 V for dimeric ferulic acids. Phenolic acid monomers were identified by comparison of their retention time with outer standards and their unique mass fragmentations. Ferulic acid dehydrodimers were identified by comparing their HPLC eluting sequence and HPLC–MS/MS fragment ion pattern with the available literature (Bauer, Harbaum-Piayda, & Schwarz, 2012; Callipo et al., 2010; Dobberstein & Bunzel, 2010; Garcia-Conesa, Plumb, Waldron, Ralph, & Williamson, 1997). The quantification of phenolic acids in alkaline extracts was accomplished by using the standard curves generated by properly diluted phenolic acid standards. Ferulic acid dehydrodimers were quantified using *trans*-cinnamic acid as a standard.

2.5. Total phenolic content (TPC) determination

Total phenolic content (TPC) of WG and DF alkaline extracts were determined by Folin–Ciocalteu method (Singleton & Rossi, 1965) as modified by Gao, Wang, Oomah, and Mazza (2002). Briefly, a 0.2 mL of appropriately diluted extract was added to 1.5 mL of freshly made 10-fold diluted Folin–Ciocalteu reagent. After 15 min equilibration, the mixture was neutralized with 1.5 mL of sodium carbonate (60 g/L), mixed well by a vortex, and the test tube sealed with paper film. After 90 min reaction in the dark, the absorbance of the mixture was measured at 725 nm with a UV–vis spectrophotometer against 50% MeOH as a blank. Ferulic acid (0 to 200 μg/mL) was used as a standard and the results were expressed as milligrammes of ferulic acid equivalent (FAE) per gram of sample (mg FAE/g sample).

2.6. Antioxidant activity (AOA) evaluation

Antioxidant activity (AOA) of WG and DF alkaline extracts was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity assay (Brand-Williams, Cuvelier, & Berset, 1995) with some modifications. Briefly, a 0.1 mL of appropriately diluted extract was combined with 3.9 mL of freshly made DPPH radical solution (60 μmol/L, prepared in 95% methanol). The absorbance was monitored at 515 nm. The absorbance of the extract with DPPH was measured at t = 60 min (60 minutes of incubation with DPPH at room temperature and in the absence of light) and described as A_{sample}. The absorbance of the blank (95% methanol) with DPPH was measured at t = 0 min (immediately after the addition of DPPH) and described as $A_{control}$. The DPPH radical scavenging capacity (%) of both alkaline extracts and trolox standard was calculated according to the equation: $(1 - A_{sample}/A_{control}) \times 100\%$. A standard curve was generated based on trolox concentrations (25 to 500 μmol/L) and its DPPH radical scavenging activity (%). According to the equation of the standard curve and the DPPH radical scavenging activity (%) of extracts, the AOA of extracts was further expressed as micromole of trolox equivalents (TE) per gram of sample (μmol TE/g sample).

2.7. Statistical analysis

The results were reported as mean ± standard deviation (SD). Data were analysed by a one-way analysis of variance (ANOVA) test