Enhancing antioxidant activity and antiproliferation of wheat bran through steam flash explosion

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Revised: 26 May 2016 / Accepted: 2 June 2016 / Published online: 22 July 2016
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Abstract The effect of steam flash explosion (SFE), a green processing technology, on the phenolic composition, antioxidant activity and antiproliferation to HepG2 of wheat bran was investigated. Moderate SFE treatment significantly enhanced the total soluble phenolic content of wheat bran. After SFE pretreatment, the free and conjugated ferulic acid content in the wheat bran were significantly increased. Antioxidant activities of SFE treated wheat bran were higher than those untreated wheat bran. The cellular antioxidant and antiproliferative activities of SFE treated wheat bran were also significantly ameliorated. It was suggested that SFE pretreatment could be applied to release the bound phenolic compounds and enhance the antioxidant activities and antiproliferative activities of wheat bran.

Keywords Steam flash explosion · Wheat bran · Phenolics · Cellular antioxidant activity · Antiproliferative activity

Introduction

The pathology of many chronic and degenerative diseases involves cellular and molecular damages caused by reactive oxygen species and free radicals (Zhang et al. 2015). It

is believed that the diet can significantly influence the overall health and quality of life (Ussher et al. 1995). Diet rich in natural antioxidants can reduce the incidence of human diseases such as cancers, type 2 diabetes, cardiovascular diseases, and other health problems related with advancing age (Liu 2003). There is a great deal of interest in developing functional foods rich in bioavailable antioxidants with health promoting potential (Lobo et al. 2010). Phenolic compounds widely spread in plants, account for an integral part of our diet with health protective roles, such as antioxidant, anticarcinogenic, antimutagenic, anti-inflammatory, antimicrobial bioactivities and so forth (Heleno et al. 2015; Sevgi et al. 2015).

Wheat bran is the main byproduct of flour milling industry. For its wide source and high phenolics content, the preparation of antioxidants from wheat bran was widely explored. It was reported that the phenolic compounds in wheat bran mainly exist in insoluble bound form and link to celluloses and hemicelluloses, which made low yield with conventional solvent extraction. So many pretreatment methods, such as mechanical crush, chemical and enzymatic hydrolysis have been employed to release bound phenolics from celluloses or hemicelluloses (Dziadas and Jelen 2016; Kim et al. 2006; Verma et al. 2009), which are time-consuming and harmful to the environment.

Steam flash explosion (SFE) is an efficient, economical and environmentally friendly processing method to destroy the structural components of plant components, such as cellulose, hemicelluloses and lignin by using a short-time steam cooking at high temperatures and pressures, rapidly releasing the pressure to around in a very short time (0.00875 s), and instantly cooling the materials to room temperature (Yu et al. 2012). It was reported that SFE could effectively release bound phenolic compounds and enhance the antioxidant capacity of barley bran (Gong

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et al. 2012). And Guo et al. (2015) found that SFE could change phytate into low inositol phosphate salts. To the best of our knowledge, there was no report about the effect of SFE on the bioactivities of wheat bran. In view of this, the alteration of SFE on the phenolic composition of wheat bran was investigated. And the chemistry and cellular antioxidant activities and the antiproliferation activity to HepG2 of wheat bran were also evaluated.

Materials and methods

Materials and chemicals

The wheat bran (2014 production, moisture content 10.33 %) was provided by New Land Group (Xinxiang, China). Ferulic acid, quercetin, gallic acid, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Dichlorofluorescein diacetate (DCFH-DA), Folin-Ciocalteu reagent, linoleic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azobis-amidinopropane (AAPH) were purchased from Sigma (St. Louis, MO). HepG2 human liver cancer cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Williams' medium E (WME), epidermal growth factor, heparin, insulin, and other cell culture reagents were purchased from Gibco U.S. Biotechnology Co. Other chemicals were of analytical grade.

Optimization of steam flash explosion (SFE) conditions

Steam flash explosion was performed on a QBS-80 batch steam explosion apparatus (Hebi Steam Explosion Research Center, Xinxiang, Henan, China), which consisted of a high pressure vessel (400 mL), a steam generator, a material tank, a receiver and a rapid-opening ball valve (Yu et al. 2012). About 80 g of wheat bran were placed inside a high pressure vessel, treated at 0.5, 1.5 and 2.5 MPa for 30 or 90 s by saturated steam from a steam generator, and then terminated by explosive decompression, which was controlled by a rapid-opening ball valve. The treated wheat bran was collected, freeze-dried (Alpha 1-2LD plus, Christ, Germany) and ground for further study.

Determination of total soluble phenolic content (TSPC)

The treated or untreated wheat bran powder (2 g) was ultrasonic extracted five times with 30 mL of 80 % methanol for 20 min at room temperature. After centrifugation at 3000 rpm for 10 min, the supernatant was combined and diluted to 250 mL by 80 % methanol to determine TSPC.

TSPC measurement was carried out according to the Folin-Ciocalteu method (Xu et al. 2007). Briefly, 0.3 mL of the above sample solution was diluted to 10 mL with distilled water, mixed with 0.5 mL of Folin-Ciocalteu phenol reagent. The reaction mixture was shaken vigorously. After 5 min, 5 mL of 5 % Na_2CO_3 solution was added to the mixture. The distilled water was added immediately to make a final volume of 25 mL. The solution was allowed to stand for 90 min. Then, the absorbance was read at 750 nm versus the prepared blank. The TSPC of the wheat bran was expressed as gallic acid equivalents (GAE) milligrams per gram of DW.

Preparation of the extract from wheat bran

In order to investigate the effect of SFE pretreatment on the phenolic composition, antioxidant activities and antiproliferative activity of wheat bran, the wheat bran were pretreated with steam flash explosion at optimal condition (2.5 MPa, 30 s). Both the extracts of treated or untreated wheat bran were prepared by the following method. Briefly, the wheat bran (30 g) was extracted three times with 600 mL of 80 % ethanol at 70 °C for 1 h and filtered under vacuum. The filtrate was collected and freeze-dried (Alpha 1-2LD plus, Christ, Germany), the obtained extract was weighted to calculate extract yield and used for the following assays.

Determination of ferulic acid content

The ferulic acid content of the extract was determined according to the previous method with slight modifications (Kim et al. 2006; Xu et al. 2007). The extract (0.5 g) was extracted three times with 80 mL of diethyl ether/ethyl acetate (1:1, v/v) at room temperature. The ether/ethyl acetate extract was filtered and evaporated to dryness under vacuum at 40 °C and solved into 10 mL of methanol to analyze the content of free ferulic acid. The residue was treated by alkaline hydrolysis (4 M NaOH, 20 mL) for 4.5 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 using 6 M HCl, ferulic acid was released from the soluble ester and extracted as described above. The content of ferulic acid in the above sample solutions were determined by an Agilent 1260 HPLC system (Santa Clara, CA). The separation was performed on a DIKMA C18(2) column (250 mm × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile (solvent A) and 1 % acetic acid (solvent B). The flow rate was set at 1 mL/min and the column temperature was at 30 °C. Gradient elution was performed as follows: 0–30 min, 100–88 % solvent B; 30–60 min, 88–50 % solvent B; 60–70 min 50–0 % solvent B; 70–80 min, 0–100 % solvent B. The quantification of ferulic acid was

based on the external standard method by using Agilent OpenLAB ChemStation software (Santa Clara, CA).

DPPH radical scavenging assay

DPPH radical scavenging assay was carried out according to the previous method with slight modifications (Wang et al. 2015). Briefly, 2 mL of DPPH solution (0.1 mM, in ethanol) was mixed with 2 mL of the extract dissolved in ethanol at different concentrations (0.2–1.0 mg/mL), shaken and incubated in the dark at room temperature for 30 min. Then, its absorbance at 517 nm against ethanol was measured. The control containing ethanol instead of the extract, and the blank containing ethanol instead of DPPH were also measured. The DPPH radical scavenging activity was calculated as the follow equation.

DPPH scavenging activity

$$= \frac{\text{Abs. of control} - (\text{Abs. of Sample} - \text{Abs. of blank})}{\text{Abs. of control}} \times 100\%$$

ABTS radical scavenging assay

ABTS radical scavenging assay was carried out according to the previous method with slight modifications (Zhu et al. 2011). The ABTS radical cation solution was obtained as follows: 100 mL of ABTS (7 mM) was mixed with 1.75 mL of potassium persulphate (140 mM) and then incubated in the dark at room temperature for 12 h. The working solution was prepared by diluting the previous solution with phosphate buffer (0.05 M, pH 7.4) until the absorbance at 734 nm was 0.70 ± 0.02 . 2.85 mL of working solution was mixed with 150 μ L of the extract dissolved in 80 % methanol at different concentrations (0.2–1.0 mg/mL), shaken and incubated in the dark at room temperature for 10 min. Then, its absorbance at 734 nm against ethanol was measured. The control containing 80 % methanol instead of the extract was also measured. The ABTS radical scavenging activity was calculated as the follow equation.

ABTS scavenging activity

$$= \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100\%$$

Reducing power assay

Reducing power assay was carried out according to the previous method with slight modifications (Liu et al. 2013). The extract dissolved in 80 % methanol at different concentrations (1.0–5.0 mg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % $\text{K}_3\text{Fe}(\text{CN})_6$. The mixture solution was incubated at 50 °C and

rapidly cooled after 20 min. 2.5 mL of 10 % trichloroacetic acid was added to the mixture, then centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % FeCl_3 , and the absorbance was measured at 700 nm after 10 min. The blank containing 80 % methanol instead of the extract was also measured. The increased absorbance of mixture solution indicated reducing power.

Antioxidant activity in a linoleic acid system

The antioxidant activity in a linoleic acid system was determined according to the previous method with slight modifications (Sakanaka et al. 2004). The 200 μ L of the extract dissolved in 80 % methanol at different concentrations (2.0, 4.0, 8.0 mg/mL) was mixed with 4 mL of phosphate buffer (0.05 M, pH 7.0), 2 mL of 2.5 % linoleic acid dissolved in ethanol and 4 mL of distilled water. The mixture was incubated in the dark at 40 °C for 30 min. Then the degree of lipid peroxidation was measured every 12 h by the ferric thiocyanate method. Briefly, 100 μ L of the sample solution was mixed with 8 mL of 75 % ethanol, 100 μ L of 30 % ammonium thiocyanate and 100 μ L of 20 mM FeCl_2 (dissolved in 3.5 % hydrochloric acid). The absorbance of the mixture was measured at 500 nm. The control containing 80 % methanol instead of the extract was also measured.

Cellular antioxidant activity (CAA) assay

The CAA assay of the extract was carried out according to the previous method (Chen et al. 2015). Briefly, the HepG2 cells in the growth medium was seeded in a 96-well microplate and incubated at 37 °C for 24 h. Then the growth medium in the wells was removed and the cells were also washed with phosphate buffer solution (PBS). 100 μ L of medium containing the extract and 50 μ M dichlorofluorescein diacetate (DCFH-DA) was added to the wells and removed after 1 h. Then, the cells were pre-treated with the sample before the AAPH (600 μ M) was added (PBS wash protocol), or pretreated with the sample and AAPH (no PBS wash protocol). The fluorescent data of each well was recorded to determine the EC_{50} values with excitation and emission wavelengths at 485 and 535 nm respectively. And the EC_{50} values were converted to CAA values, which were expressed as micromoles of quercetin equivalents (QE) per 100 g of the extract.

Antiproliferative assay

The antiproliferative assay was performed by using the method described by Felice (Felice et al. 2009). Briefly, the HepG2 cells in the growth medium was seeded in a 96-well

microplate and incubated at 37 °C for 4 h. The growth medium was removed and the fresh medium containing different concentrations of the extract was added to wells. The control containing the growth medium instead of the extract was also made. After 72 h of incubation at 37 °C, the cell viability was determined by the methylene blue method and the EC_{50} value was also evaluated and expressed as milligrams of extract/mL.

Statistical analysis

The data obtained in this study were expressed as the mean of three replicate determinations plus or minus the standard deviation (SD). Statistical comparisons were made with Student's test. p values <0.05 were considered to be significant.

Results and discussion

Fitting the model of SFE

It was reported that steam explosion led to the hydrolysis of glycosidic bonds in the hemicelluloses and celluloses or the depolymerization of lignin (Kim et al. 2006). And the destructive degree depended on the type and physical accessibility of the raw material used. In this study, the SFE treatment at different pressures and times were performed and the total soluble phenolic content (TSPC) of wheat bran was evaluated (Table 1). The SFE treatment significantly improved the TSCP. Similar increase in phenolics yields after steam explosion were reported earlier (Conde et al. 2009). And with increases of treatment pressure and time, TSCP increased progressively steam pressure and time of 2.5 MPa and 30 s, respectively, reduced. Further increases in treatment severity led to the decrease of TSPC, probably due to thermal decomposition of phenolics. The extract yield of wheat bran treated at 2.5 MPa and 30 s was 32.30 % and higher than that of untreated wheat bran (16.09 %), which coincided with the previous report that the SFE could destroy the tight plant texture to enhance the extract yield (Gong et al. 2012).

Ferulic acid content

Ferulic acid was reported to be the predominant phenolic acid in wheat bran and bran-derived samples, which existed in insoluble bound form by linking with polysaccharides in cell walls. In order to evaluate the release of ferulic acid from wheat bran treated by the optimal SFE condition, the free and conjugated ferulic acid in the extract was measured (Table 2). Untreated wheat bran extract showed significantly high conjugated ferulic acid than that of free

Table 1 Effect of SFE on TSPC of wheat bran

Treatment	TPC (mg GAE/g DW)*
Untreated	3.834 ± 0.191 ^a
0.5 MPa, 30 s	3.791 ± 0.144 ^a
0.5 MPa, 90 s	3.916 ± 0.144 ^a
1.5 MPa, 30 s	8.000 ± 0.760 ^b
1.5 MPa, 90 s	19.834 ± 0.505 ^c
2.5 MPa, 30 s	27.709 ± 0.833 ^d
2.5 MPa, 90 s	21.041 ± 0.505 ^c

* Values in the column with no letters in common are significantly different ($p < 0.05$)

Table 2 Effect of SFE on the content of free and conjugated ferulic acid in the extract (μg/g of dry weight)

	Free ferulic acid*	Conjugated ferulic acid*
Untreated	Trace ^a	348.77 ± 11.59 ^a
Treated at 2.5 MPa, 30 s	97.79 ± 14.38 ^b	2391.56 ± 325.84 ^b

* Values in the column with no letters in common are significantly different ($p < 0.05$)

ferulic acid, which was coincide with the previous reports that ferulic acid in wheat bran existed in the bound form (Kim et al. 2006). After treated by SFE, free and conjugated ferulic acid content in extracts were significantly increased. The conjugated ferulic acid content of extracts from SFE treated bran was near seven times of that of the untreated (Fig. 1). The increase of the soluble ferulic acid could attribute to the destruction of their glycosidic linkage and the breakage of hemicelluloses or celluloses caused by SFE, which may have led to the changes of bioactive of the wheat bran extract (Gong et al. 2012; Verma et al. 2009).

Fig. 1 HPLC profile of the extracts hydrolyzed by alkaline

Fig. 2 Chemical antioxidant activities of the extracts from wheat bran untreated and treated with SFE

Fig. 3 Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation over time by the extracts from wheat bran untreated and treated with SFE

Table 3 Cellular antioxidant activity of the extracts from SFE treated and untreated wheat bran

Extract	EC ₅₀ value (μg/mL)*		Cellular antioxidant activity (μmol of quercetin/100 g of extract)*	
	No PBS wash	PBS wash	No PBS wash	PBS wash
Untreated	5038.70 ± 318.87 ^a	7312.96 ± 57.49 ^a	76.35 ± 4.68 ^a	73.68 ± 0.58 ^a
Treated at 2.5 MPa, 30 s	895.78 ± 22.12 ^b	4213.35 ± 199.40 ^b	428.56 ± 10.68 ^b	128.07 ± 6.18 ^b

* Values in the column with no letters in common are significantly different ($p < 0.05$)

Chemistry antioxidant activities

In this study, four commonly accepted assays were employed to compare the antioxidant activity of extracts of the SFE treated and un-treated bran (Fig. 2). Both DPPH and ABTS radical scavenging assays can be used to evaluate many samples in a short period and to detect active ingredients at low concentrations. It was found that the radical scavenging activities of the treated were stronger than those of the un-treated (Fig. 2a, b). For example, the DPPH and ABTS radical scavenging activities of the treated extract at the concentration of 0.8 mg/mL were 84.17 and 50.27 %, respectively. The DPPH and ABTS radical scavenging activities of the untreated extract were only 30.60 and 10.88 % respectively. Reducing power assay reflex the iron (III) to iron (II) reduction capacity of the antioxidant. Treated and untreated bran extracts showed the reducing power in the range of 1–5 mg/mL (Fig. 2c). And the performance of the treated was superior to that of the untreated. The antioxidant activities of the extracts were also determined by peroxidation of linoleic acid using the thiocyanate method (Fig. 2d). In this assay, the formed peroxides can oxidize Fe^{2+} to Fe^{3+} . And Fe^{3+} can combine SCN^- to form a complex with a maximum absorbance at 500 nm. High absorbance means the poor antioxidant of a sample in the emulsion system. It was found that the absorbance of the untreated extract at low concentrations (2 and 4 mg/mL) were higher than that of control, which suggested that it failed to exhibit the antioxidant activity. At 8 mg/mL, the antioxidant activity of the no-treatment was only higher than that of the treatment at 2 mg/mL. And the treatment at all the test concentrations showed the strong antioxidant activity in a dose-dependent manner. In the previous reports, it was found that SFE improved the antioxidant activity of the extract (Kurosumi et al. 2007; Sun et al. 2014), which coincided with present results.

Cellular antioxidant activity

For most chemistry antioxidant activity assays, they were performed at nonphysiological conditions. So they could not reflect the bioavailability, uptake, and metabolism of the

samples. In view of this, a cellular antioxidant activity (CAA) assay was developed to determine the antioxidant activity of bioactive compounds in human hepatocarcinoma HepG2 cells with dichlorofluorescein as a probe. And the decrease of cellular fluorescence can indicate the in vivo antioxidant activity of the samples. In this study, the antioxidant activity of the extracts was further evaluated by the CAA assay. It was found that both the extracts could inhibit the peroxy radical-induced oxidation of DCFH to DCF in HepG2 cells (Fig. 3). The EC₅₀ values of the no-treatment and treatment extracts in no PBS wash protocol were 5038.70 ± 318.87 and 895.78 ± 22.12 μg/mL, respectively. The treated extract showed the higher antioxidant activity than untreated. The PBS wash protocol focused more on the intracellular antioxidant activity, the treatment extracts still exhibited the higher bioactivity in this protocol (Table 3). By comparing the EC₅₀ values of quercetin, the CAA values of the extract could be expressed as micromoles of quercetin/100 g of extract (Table 3), which were negatively correlated with the EC₅₀ values. Regardless of the protocol and expression method used, the treatment extract exhibited higher antioxidant activity, which suggested that SFE could improve the cellular antioxidant activity of wheat bran.

Fig. 4 Percent inhibition of human HepG2 liver cancer cell proliferation by the extracts from wheat bran untreated and treated with SFE

Antiproliferative activity

The antiproliferation activities of the no-treatment and treatment extracts against liver cancer cell HepG2 are shown in Fig. 4. Both the extracts could inhibit the cell proliferation a dose-dependent manner. The antiproliferative activity of the treatment extract (EC_{50} value, 3.12 ± 0.16 mg/mL) was higher than that of the no-treatment extract (EC_{50} value, 13.62 ± 0.13 mg/mL), which was consistent with CAA result and could be explained by the higher content of soluble phenolic contents, especially soluble ferulic acid (Tan et al. 2015; Chandrasekara and Shahidi 2011).

Conclusions

The SFE treatment at 1.5 or 2.5 MPa could significantly improved the total soluble phenolic content (TSPC) of wheat bran. The increase of the free and conjugated ferulic acid content in the extract was observed after treatment. As a result, antioxidant activities of the extract from treated wheat bran were higher than those of untreated. The cellular antioxidant and antiproliferative activities of the treated wheat bran were significantly enhanced. Results showed that the wheat bran treated by SFE has antioxidant activity and nutraceuticals preparations. SFE technology can be also used to improve the phenolic composition and bioactivities of other plant foods or byproducts.

Acknowledgments We gratefully acknowledge supports of this research by the National Natural Science Foundation of China (Nos. 31571782 and 31571881) and the Program for Science and Technology Innovation Talents in Universities of Henan Province (14HASTIT019).

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