Journal of Cereal Science 68 (2016) 116–121

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Enhanced antioxidant activity of rice bran extract by carbohydrase treatment

Sung-Min Kim, Seung-Taik Lim*

Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, 5-1 Anam-dong, Sungbuk-ku, Seoul 136-701, South Korea

ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form
15 January 2016
Accepted 19 January 2016
Available online 22 January 2016

Keywords:
Rice bran
Carbohydrase
Phenolic compound
Antioxidant activity
Phenolic acid

ABSTRACT

Rice bran (RB) was treated with different carbohydrases (Viscozyme, Termamyl, Celluclast, AMG, Ultraflo, and Pentopan), and then aqueous alcoholic extracts (50% ethanol) from the treated RB were examined for their phenolic compositions and antioxidant activities (radical scavenging activity and ferrous reducing activity). All the carbohydrases tested induced significant increases in ferric reducing power (1.5–3.3 times). Among the enzymes tested, Pentopan which was active in pentosan hydrolysis appeared to be most effective in increasing the antioxidant activity. Celluclast, Ultraflo, and Pentopan appeared more effective in increasing the total phenolic content (TPC) as well as radical scavenging activity than other enzymes. These enzymes increased the amount of extractable phenolic acids by 2.5–3.0 times, especially for ferulic, *p*-coumaric, vanillic, and *p*-hydroxybenzoic acids. Enzymatic hydrolysis of cell wall polysaccharides in RB could be used as an effective procedure for raising the amount of extractable phenolic acids and thus increasing the antioxidant activity of RB extract.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Rice bran (RB), a byproduct from the milling of brown rice, contains a variety of nutritive substances and health-promoting phytochemicals including γ-oryzanol, tocotrienols, phytosterols, policosanol, squalene, phytic acid, and various phenolic compounds (Butsat and Siriamornpun, 2010). The phenolic compounds in RB have been extensively investigated and reported to exhibit diverse bioactivities, such as antioxidative, antimutagenic, anticarcinogenic, antimicrobial, and anti-inflammatory properties (Alrahmany et al., 2013; Nam et al., 2005). Additionally, those phenolic compounds play an important role in preventing some chronic diseases, such as diabetes, cardiovascular disease, and certain cancers (Balasubashini et al., 2003; Hudson et al., 2000; Jung et al., 2007).

The RB phenolic compounds may be categorized into two groups: free phenolics which may be readily extracted from RB, and bound phenolics which are covalently bound to cell wall components (such as cellulose, hemicellulose, pectin, lignin, structural proteins, etc.) (Adom and Liu, 2002; Zhou et al., 2004). Adom and Liu (2002), and Zhou et al. (2004) reported that more than 70% of the total phenolics present in RB exist as the insoluble (bound) form and have a higher antioxidant capacity than free phenolics. The bound phenolics in plants are not easily extracted in aqueous or organic solvents. To improve the extractability and thus the bio–functional activity, chemical degradation or disruption of plant cell wall matrices by acid or base hydrolysis have been commonly practiced (Gómez-García et al., 2012; Li et al., 2006). However, those treatments are not desired to be applied in commercial scale because the degradation is not selective to the phenolic linkages and often causes a viscosity increase due to fiber dissolution inhibiting the phenolic extraction (Jodayree et al., 2012). On the other hand, enzymes can depolymerize the cell wall polysaccharides and/or hydrolyze linkages between phenolic compounds and cell wall matrices, resulting in weakening the cell wall and improving the extractability of the phenolic compounds. Successful applications of cell wall degrading enzymes for the extraction of phenolic compounds have been reported by several researchers for a variety of plant including apple peel, citrus peel, black currant pomace, *Thymus vulgaris*, *Ginkgo biloba* leaves, finger millet, oat bran, wheat bran, and red algae (Alrahmany and Tsopmo, 2012; Alrahmany et al., 2013; Cerda et al., 2013; Chen et al., 2011; Gómez-García et al., 2012; Kapasakalidis et al., 2009; Kim et al., 2005; Li et al., 2006; Moore et al., 2006; Wang et al., 2010; Yadav et al., 2013). However, no study has been reported on the utilization of carbohydrases to increase the extraction of phenolics from RB.

* Corresponding author.
E-mail address: limst@korea.ac.kr (S.-T. Lim).

http://dx.doi.org/10.1016/j.jcs.2016.01.006
0733-5210/© 2016 Elsevier Ltd. All rights reserved.

The objectives of this study were to determine the effects of the treatment of RB with different commercially available carbohydrases (Viscozyme, Celluclast, Termamyl, AMG, Ultraflo, and Pentopan) on the extraction of total phenolic compounds and the changes in antioxidant activities of RB extracts by following the methods using DPPH and FRAP. Furthermore, the composition of individual phenolic acids of RB extracts were also identified by reverse-phase high-performance liquid chromatographic (HPLC).

2. Materials and methods

2.1. Materials

The rice bran (*Oryza sativa* L., Japonica type) used in this study was a byproduct from the milling of brown rice for the production of white rice which was provided by Cheiljedang Co. (Seoul, Korea). Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and the standard chemicals of phenolic acids were purchased from Sigma–Aldrich Company (St. Louis, MO). HPLC-grade methanol, acetonitrile, and acetic acid were purchased from Thermo Fisher Scientific Inc. (Waltham, MA). All other chemicals and reagents were of analytical grade. The carbohydrolases were commercial products purchased from Novozymes A/S (Bagsvaerd, Denmark) and their characteristics are summarized in Table 1. The pH and temperature for the hydrolysis was selected based on the optimum values provided by the company and the preliminary experiments (data not shown).

2.2. Enzymatic hydrolysis and extraction

The enzyme treatments of RB were performed according to the method of Alrahmany and Tsopmo (2012) and Wang et al. (2010) with some modifications. The RB (7 × 2 g solids) was defatted by dispersing in *n*-hexane (1:6, w/v) for 2 h at room temperature with a magnetic stirrer. The mixture was then filtered (Whatman No. 1), and the RB residue was dried overnight in a fume hood. To each defatted RB, 30 mL of 50 mM sodium acetate buffer of selected pH value (4.6 for Viscozyme, AMG, and Ultraflo; 5.0 for Celluclast and Pentopan; 6.0 for Termamyl) was added, and then 100 μL of each enzyme solution was added to the dispersion based on the reported method. The hydrolysis was performed by stirring at 50 °C for 12 h in a thermostatically controlled water bath (150 rpm). The enzymatic hydrolysis was terminated by boiling the dispersion for 10 min and thereafter immediate cooling in an ice bath. The pH was then adjusted equally to 7.0 by adding 1 M NaOH. For further extraction of phenolics, 30 mL of ethanol was added to the dispersion to make a 50% alcohol solution, and then the mixture was stirred at 50 °C for 2 h. The extract was recovered by centrifuging at 2500 *g* for 15 min and the supernatant was filtrated (Whatman paper No. 1). The solvent was removed using a rotary evaporator (Eyela NN series, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 40 °C, freeze-dried, and stored at −20 °C until further analyses. A control extract was also prepared out by following the same procedure without enzyme.

2.3. Extraction yields and sugar content

The extraction yield of solids was defined as the weight percentage of the extractable solids with respect to the original RB solids. The hydrolysis level was monitored by determining the total and reducing sugar contents in the extracts by the phenol-sulfuric acid method (DuBois et al., 1956) and dinitrosalycilic acid (DNS) method (Miller, 1959), respectively. The results were expressed as milligrams of glucose equivalents per gram of sample dry weight (mg/g).

2.4. Determination of total phenolic content (TPC)

The total phenolic content (TPC) in the RB extracts was determined based on the Folin-Ciocalteu method described by Adom and Liu (2002) with slight modifications. The freeze-dried extract obtained from 2 g of RB solids was dissolved in 50% ethanol solution (20 mL), and then an aliquot of the extract solution (200 μL) was mixed with 10-fold diluted Folin-Ciocalteu reagent (800 μL). The mixture was allowed to stand at room temperature for 5 min, and then a saturated Na_2SO_3 solution (75 g/L, 2 mL) was added to the mixture. After standing for 2 h at room temperature in the dark, the absorbance of the resulting solution was measured at 765 nm using UV-spectrophotometer (BioMate 3S, Thermo Scientific, USA). The TPC of the extracts was calculated and expressed as milligrams of gallic acid equivalents per gram of sample dry weight (mg GAE/g).

2.5. DPPH radical scavenging activity

The antioxidant activity of the extracts was determined using the DPPH radical scavenging assay described by Butsat and Siriamornpun (2010) with slight modifications. Briefly, 500 μL of the water-ethanol extract solution (dissolved in 50% aqueous ethanol) was added to 0.075 mM DPPH solution in ethanol (500 μL) and the mixture was vortexed thoroughly. The reaction solution was carried out at room temperature for 30 min in the dark. Then, the absorbance of the reaction mixture was measured spectrophotometrically at 517 nm. A standard curve was prepared by measuring the reduction in the absorbance of the DPPH solution at different concentrations of Trolox solutions. The DPPH scavenging activity was expressed as micromoles of Trolox equivalents per gram of sample dry weight (μmol TE/g).

Table 1
Characteristics, sources, and optimum conditions of commercial carbohydrases.

Enzymes[a]	Characteristics[b]	Sources	Optimum conditions[b]	
			pH	Temp. (°C)
Viscozyme L	A multi enzyme complex (containing arabanase, cellulase, β-glucanase, hemicellulase, and xylanase)	*Aspergillus aculeatus*	3.5–5.5	40–50
Terrmamyl 120L	Heat-stable α-amylase	*Bacillus licheniformis*	5.5–6.0	50–60
Celluclast 1.5 L	Cellulase	*Trichoderma reesei*	4.5–6.0	40–60
AMG 300 L	Exo-1,4-α-D-glucosidase	*Aspergillus niger*	4.5	50–60
Ultraflo L[c]	Heat stable multi-activeβ-glucanase	*Humicola insolens*	4.5–7.0	40–60
Pentopan 500 BG[d]	Endo-1-4-β-xylanase	*Thermomyces lanuginosus*	4.0–6.0	45–55

[a] Commercial products of Novozymes A/S (Bagsvaerd, Denmark).
[b] Provided by the manufacturers.
[c] Containing extra activity: feruloyl esterase (Faulds et al., 2004).
[d] Containing extra activity: feruloyl esterase, caffeoyl esterase, and pectinase (Gioia et al., 2007).

2.6. Ferric reducing antioxidant power (FRAP) assay

The method of Butsat and Siriamornpun (2010) was used with some modifications. Briefly, the stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was warmed at 37 °C before using. The 300 μL of the solution of extracts (dissolved in 50% aqueous ethanol) was allowed to react with 1.7 mL of the FRAP solution. The absorbance was measured spectrophotometrically at 510 nm after 15 min of reaction. The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and expressed as micromoles of $FeSO_4$ per gram of sample dry weight (μmol $FeSO_4$/g).

2.7. Determination of phenolic acid compositions

The phenolic acid composition in the RB extracts was determined using an HPLC (Dionex UltiMate 3000, Thermo Fisher Scientific, Co., Ltd, MA, USA) equipped with gradient pump (LPG-3400SD), automated sample injector (WPS-3000SL), C-18 column (4.6 mm × 250 mm, 5 μm; Shiseido, Tokyo, Japan), and UV–vis detector (VWD-3100). The column was operated at 40 °C. The mobile phase consisted of solvent A (water:acetic acid, 100:1, v/v) and B (MeOH/acetonitrile/acetic acid, 95:5:1, v/v/v), and a multi-step gradient was used at a flow rate of 1.0 mL/min with an initial injection volume of 10 μL. Before analysis, all dried extracts were dissolved in 50% aqueous methanol and then filtered through a 0.2 μm syringe filter. Hydroxybenzoic acid compounds (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and syringic acid) were detected at a wavelength of 280 nm, whereas hydroxycinnamic acid compounds (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid) were determined at 325 nm.

2.8. Statistical analysis

Data from the analysis were reported as mean ± standard deviation (SD) for at least triplicate analyses of each sample. All the statistical analyses were carried out with Duncan's multiple test using SAS software system (version 9.2, SAS Institute Inc., Cary, NC). Pearson's correlation coefficient was used for the correlation analysis. The statistical significance was considered for $p < 0.05$.

3. Results and discussion

3.1. Extraction yields and sugar content

All commercial enzymes tested in this study were effective in improving the extraction of the soluble solids from RB (Table 2). Pentopan showed the highest yield (72.2%), which was approximately 2.4 times higher than that of the control (no enzyme, 29.8%), followed by Viscozyme (59.0%), Ultraflo (57.3%), and Celluclast (55.3%). Similar results regarding the increase of extraction yield by enzymatic treatments have been reported by several researchers (Alrahmany and Tsopmo, 2012; Wang et al., 2010). For example, Alrahmany and Tsopmo (2012) reported that the yield of soluble solids (extraction yield) from oat bran increased 1.6–4.4 times after treatment with carbohydrases (Viscozyme, α-Amylase, Celluclast, and Amyloglucosidase). In present study, the extraction was performed using one (50% ethanol) of aqueous alcohol solutions which are commonly used for the extraction of phenolics from plant tissues. Our results indicate that the amount of extractable compounds which might include degraded cell wall polysaccharides and released phenolic compounds (extraction yield) increased substantially by the enzymatic treatment (Table 2). The hydrolysis of polysaccharides could be monitored by measuring the amount of total and reducing sugars in the RB extracts. The total sugar content of the control sample was 134.3 mg/g, whereas those of the enzyme-treated samples were higher ranging from 197.9 to 333.4 mg/g (Table 2). Pentopan and Termamyl showed the greatest increases in total sugar content (333.4 and 328.2 mg/g, respectively). The effect of enzymatic treatment on the total sugar contents appeared similar to that on the total solid contents in the extracts, which indicates that the increases in extraction yield were from the hydrolysis of the cell wall polysaccharides. Compared to the untreated RB sample, the reducing sugar contents increased in all the samples treated with carbohydrases and exhibited a similar trend to that of the total sugar contents (Table 2). The Celluclast-treated sample (139.6 mg/g) exhibited the smallest increase, whereas the Pentopan-treated sample (303.2 mg/g) showed the largest increase. Alrahmany and Tsopmo (2012) reported that the reducing sugar contents in oat bran increased 6–10 times after carbohydrase treatments. Kapasakalidis et al. (2009) observed that the amount of reducing sugar increased from 3.86 mg/mL to 6.13 mg/mL in black currant by cellulase treatment.

3.2. Total phenolic contents (TPC)

The total phenolic contents (TPC) of the control and carbohydrase-treated RB samples are shown in Table 2. The TPC of the control in this study (6.5 mg GAE/g) was similar to the values of five rice cultivars grown in southern China (6.4–7.8 mg GAE/g) (Ti et al., 2014). However, there was a study reporting that a Thai rice variety contained a relatively low amount of TPC (2.5–2.7 mg GAE/g) (Butsat and Siriamornpun, 2010). This may be attributable to differences in rice varieties, growing conditions, degree of milling, and extraction conditions. Among those enzymes, Pentopan was the most effective in increasing the TPC (13.51 mg GAE/g). The highest levels of extractable solids and reducing sugars for the Pentopan-treated sample imply the highest activity of this enzyme in the cell wall hydrolysis. The degradation of cell wall matrix could improve the permeability and porosity of cell walls, thus readily leaking out the internal components including some phenolic compounds (Cerda et al., 2013; Chen et al., 2011). Pentopan

Table 2
Extraction yield, total/reducing sugar, and total phenolic content in carbohydrase-treated rice bran samples.

Samples	Extraction yield (%)	Total sugar (mg/g)	Reducing sugar (mg/g)	TPC (mg GAE/g)
Control	29.8 ± 0.8^{e}	134.3 ± 8.5^{e}	62.3 ± 3.0^{f}	6.5 ± 0.4^{e}
Viscozyme	59.0 ± 1.9^{b}	268.2 ± 6.6^{b}	248.3 ± 7.2^{b}	9.9 ± 0.2^{d}
Termamyl	44.8 ± 2.2^{d}	328.2 ± 7.1^{a}	223.3 ± 9.8^{c}	6.9 ± 0.3^{e}
Celluclast	55.3 ± 1.4^{b}	197.9 ± 7.8^{d}	139.6 ± 7.7^{e}	10.7 ± 0.1^{c}
AMG	50.6 ± 1.8^{c}	274.5 ± 1.0^{b}	207.8 ± 5.0^{c}	7.1 ± 0.4^{e}
Ultraflo	57.3 ± 0.1^{b}	213.8 ± 7.8^{c}	173.7 ± 7.8^{d}	11.8 ± 0.3^{b}
Pentopan	72.2 ± 2.7^{a}	333.4 ± 2.4^{a}	303.2 ± 11.1^{a}	13.5 ± 0.3^{a}

Different letter indicate the differences with statistical significance ($p < 0.05$).

contains a xylanase as the major enzyme, but also additional enzymes such as pectinase, feruloyl esterase, and caffeoyl esterase (Table 1) (Gioia et al., 2007). Even though there are few researches on the fine structure and components of RB, it can be assumed that phenolics are linked to hemicelluloses, such as xylan and arabinoxylan. It is already reported that phenolic compounds found in oat bran cell wall is bound to the xylopyranose polymer and those in wheat bran are esterified to the C-5 hydroxyl group of α-L-arabinofuranosyl substituents (Alrahmany and Tsopmo, 2012; Moore et al., 2006). In addition, the esterase in Pentopan could hydrolyze the ester linkages between lignin and phenolic acids such as ferulic and caffeic acids (Bagger-Jørgensen and Meyer, 2004). Furthermore, the pectinase in Pentopan could hydrolyze the pectin in external layer of cell wall and assist the release of phenolic acids (Cerda et al., 2013). Ultraflo also contains additional enzymes hydrolyzing the ester linkages between cell wall polysaccharides and phenolic acids, which might increase the level of TPC (Table 2) (Faulds et al., 2004; Yadav et al., 2013). However, the reducing sugar content in the Ultraflo-treated RB extract did not seem to increase much, which implies that the hydrolysis of cell wall polysaccharides by Ultraflo was not as significant as that by Pentopan. Overall, data suggested that the enzymes with multiple activities could be effective in the release the residual phenolic components from RB.

3.3. DPPH radical scavenging activity

The ability of antioxidants to scavenge DPPH is attributed to their hydrogen donating activity and the radical scavenging activity is often used to evaluate the antioxidant activity of natural compounds (Prior et al., 2005).

As shown in Fig. 1A, the DPPH radical scavenging activities of the control and enzyme-treated RB samples ranged from 13.8 to 24.4 μM TE/g. In particular, the Pentopan-treated sample (24.4 μM TE/g) showed the highest DPPH scavenging activity, which was approximately 1.6 times higher than that of the control sample (15.4 μM TE/g). On the whole, the results indicate that the DPPH scavenging potency of RB extracts increased by the treatment with cellulolytic- or hemicellulolytic-enzymes (Fig. 1A). Namely, except the sample treated with Viscozyme, the RB samples treated with Celluclast, Ultraflo, and Pentopan showed significantly higher DPPH scavenging activities than that of the control (Fig. 1A). The increased DPPH scavenging activity by the enzymatic treatment was due to the increased extractability of phenolic compounds (generally reported to be potent DPPH radical scavengers) from cell wall matrices as shown in Table 2. These results were in agreement with the findings reported by many researchers (Alrahmany et al., 2013; Gó;mez-García et al., 2012; Kapasakalidis et al., 2009; Kim et al., 2005; Moore et al., 2006; Wang et al., 2010): the increases in extractable phenolic content and antioxidant activity of the extracts by enzymatic treatments for various plant samples.

Meanwhile, Viscozyme-treated extract exhibited a significantly lower DPPH scavenging activity, although the TPC was higher than that of the control (Table 2 and Fig. 1A). A possible explanation for the contradictory results is that the phenolic composition in the Viscozyme-treated extract was different from that in other extracts because of different hydrolytic behaviors among the enzymes. Indeed, the phenolic compounds having different chemical structures could exhibit different degrees of scavenging capacities. For example, the phenolics having multiple hydroxyl groups generally exhibit higher scavenging potency than those having single hydroxyl group (Alrahmany and Tsopmo, 2012). In addition, other possible explanation is that the phenolics extracted by the enzymatic hydrolysis became inactivated by interacting subsequently with other constituents in the extract (such as proteins and polysaccharides), resulting in lowered scavenging effect of phenolics (Wang et al., 2010).

3.4. Ferric reducing antioxidant power (FRAP)

The FRAP assay has been used to assess the electron-transferring activity of antioxidative compounds. The reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion at low pH causes a colored ferrous-TPTZ complex in the presence of antioxidants, which increase the absorbance at 595 nm, indicating an increase in the reducing power (Benzie and Szeto, 1999).

As shown in Fig. 1B, the FRAP values of all the enzyme-treated samples were significantly higher than that of the control. The Pentopan-treated sample (68.8 μM $FeSO_4$/g) exhibited the highest FRAP value among all tested samples, whereas the Viscozyme-treated sample (30.6 μM $FeSO_4$/g) showed the lowest value. The increase in FRAP value after the carbohydrase treatment for RB

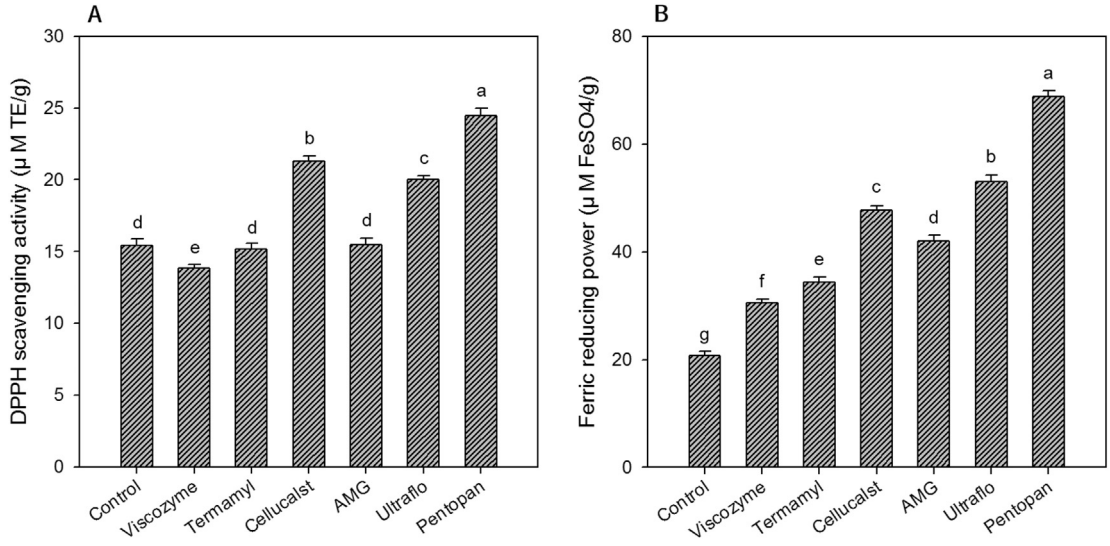

Fig. 1. DPPH radical scavenging activity and ferric reducing antioxidant power of the control and carbohydrase-treated rice bran samples. Different alphabets indicate the differences with statistical significance ($p < 0.05$).

indicates the increased release of reductones, which may be mainly phenolic compounds.

On the other hand, it was noteworthy that the RB samples treated with Termamyl and AMG did not show any significant difference in TPC compared to the control (Table 2), but higher FRAP values. Several scientists described that unnoticeable increase in phenolic compounds may result in a considerable increase in antioxidant activity (Cerda et al., 2013). In our study, it can be explained by the two possibilities. First, the degraded and soluble cell walls polysaccharides might provide an indirect effect of improving the antioxidant activity of the residual phenolic components. Second, the commercial enzymes possess more than one activity, and thus secondary enzymes would likely catalyze phenolic conversion to possibly more active compounds with different properties (Bagger-jørgensen and Meyer, 2004).

3.5. Phenolic acid composition

The eight major phenolic acids in RB samples analyzed by a HPLC system (Butsat and Siriamornpun, 2010) are shown in Table 3. The sum of individual phenolic acid contents (SPC) identified by the HPLC analysis of all the tested samples showed significant positive correlations with the TPC, DPPH scavenging activity, and ferric reducing power: $r = 0.812$, 0.916, and 0.718, respectively ($n = 7$, $p < 0.05$). It suggests that the phenolic acids were one of the major active compounds contributing to the antioxidant activity of enzymatically treated RB samples.

The SPC and phenolic acid composition in RB were substantially changed by the treatment with carbohydrase (Table 3). The SPC in the RB extracts increased by 2.5–3.0 times by the treatment with Celluclast, Ultraflo or Pentopan. It may demonstrate that the hydrolysis of cell wall polysaccharides assisted the release of phenolic acids from RB. Besides, the presence of additional enzymes active in hydrolyzing phenolic ester linkages in the commercial enzymes induced the increase of SPC (Cerda et al., 2013). Ultraflo and Pentopan were reported to contain extra-enzymes which may hydrolyze the ester linkages between phenolic acids and cell wall materials (Faulds et al., 2004; Gioia et al., 2007). On the other hand, Viscozyme and Termamyl did not affect the SPC in the RB extract, and the AMG even showed a decrease in SPC (162.9 μg/g). The relatively low values of SPC in the RB extracts treated with Termamyl and AMG agreed with the low values in TPC and antioxidant activity (Table 2 and Fig. 1). Viscozyme-treated sample, however, showed a low SPC, even though it had relatively high extraction yield and TPC (Table 2). It was hypothesized that the depolymerization of cell wall polysaccharides by this enzyme made the matrices from which individual phenolic acids were not readily released into the extract. Additional study should be done to understand the exact enzymatic action and mechanism for this phenomenon.

Alrahmany et al. (2013) reported increased free phenolic acids from oat bran by different carbohydrases (Viscozyme, Alpha-amylase, and Cellulase), which was more pronounced for ferulic and caffeic acids. Moore et al. (2006) reported that Ultraflo was the most efficient enzyme, able to convert as much as 50% of the insoluble bound ferulic acid in wheat bran to the soluble free form. As shown in Table 3, among the phenolic acids tested, ferulic acid (FA, 64.5 μg/g), vanillic acid (VA, 52.1 μg/g), *p*-coumaric acid (*p*-CA, 35.8 μg/g), and *p*-hydroxybenzoic acid (*p*-OH, 27.0 μg/g) were the major phenolic acids in the native RB (Control sample in Table 3), which was consistent with the previous report (Butsat and Siriamornpun, 2010). There were significant changes in those phenolic acids by the enzymatic treatments. The *p*-OH content was increased by the treatments with Viscozyme (1.5 times), Celluclast (2.4 times), Ultraflo (2.2 times), and Pentopan (4.9 times). The Pentopan sample especially showed the greatest increase on the VA content (5.3 times relative to the control). The *p*-CA content was increased in all the enzyme-treated samples, except for the AMG sample, and the highest increase was achieved by Pentopan (5.2 times relative to the control). The FA content was significantly increased by the Celluclast and Ultraflo treatments (225.6 and 264.2 μg/g, respectively), but decreased by Pentopan treatment (17.1 μg/g). Ferulic acid can be converted to other metabolic substances, such as 4-vinylguaiacol, 4-vinylphenol, vanillin, vanillic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and protocatechuic acid by enzymatic or microbial action (Mathew and Abraham, 2006; Tripathi et al., 2002). Therefore, the decrease of FA content and the increase in *p*-OH, VA, and *p*-CA content for the Pentopan-treated sample could be explained by the bioconversion of FA to *p*-OH, VA, and *p*-CA during the enzymatic treatment.

Based on the data in this study, the treatment of RB with commercial carboxylases successfully increased the efficiency of phenolic acid extraction from RB, not only by the enzymatic hydrolysis of the cell-wall components but also by the release of free phenolic acids. Therefore, this enzymatic treatment could be an effective process to improve the bio-function and antioxidant activity of the RB.

4. Conclusions

Rice bran (RB) is a good source of phenolic compounds, which have pronounced antioxidant activity, but the extraction of the phenolic compounds from native RB is often incomplete because of their strong linkages to insoluble cell wall polysaccharides. Commercial carbohydrate degrading enzymes, such as Celluclast, Ultraflo, and Pentopan, effectively improved the extractability of the phenolic compounds. In particular, phenolic acids, such as ferulic acid, *p*-coumaric acid, vanillic acid, and/or *p*-hydroxybenzoic

Table 3
Phenolic acid composition of carbohydrase-treated rice bran samples.[a]

	Hydroxybenzoic acid derivatives[b] (μg/g)				Hydroxycinnamic acids derivatives[b] (μg/g)				SPC[c]
	PCCA	*p*-OH	VA	SyA	CFA	*p*-CA	FA	SNA	
Control	20.5 ± 1.1^{c}	27.0 ± 1.4^{d}	52.1 ± 1.7^{f}	8.3 ± 0.3^{c}	5.9 ± 0.3^{c}	35.8 ± 1.1^{c}	64.5 ± 3.9^{c}	1.4 ± 0.0^{de}	215.4 ± 9.7^{d}
Viscozyme	5.1 ± 0.5^{f}	39.9 ± 1.2^{c}	100.9 ± 5.0^{d}	3.2 ± 0.2^{d}	5.0 ± 0.2^{d}	40.8 ± 0.9^{c}	13.2 ± 0.5^{f}	1.8 ± 0.1^{cd}	209.9 ± 8.3^{d}
Termamyl	9.6 ± 0.4^{d}	19.9 ± 0.9^{d}	77.9 ± 4.5^{e}	7.8 ± 0.4^{c}	5.3 ± 0.3^{cd}	37.4 ± 0.9^{c}	49.9 ± 4.3^{d}	1.2 ± 0.1^{e}	209.1 ± 11.8^{d}
Celluclast	24.6 ± 0.9^{b}	65.2 ± 1.9^{b}	147.1 ± 4.1^{b}	9.9 ± 0.3^{b}	8.9 ± 0.2^{b}	51.0 ± 0.9^{b}	225.6 ± 5.7^{b}	2.2 ± 0.2^{bc}	534.6 ± 5.7^{c}
AMG	8.0 ± 0.5^{e}	20.2 ± 0.5^{d}	59.2 ± 2.1^{f}	3.3 ± 0.5^{d}	5.7 ± 0.4^{cd}	25.3 ± 0.9^{d}	38.8 ± 3.4^{e}	2.4 ± 0.4^{b}	162.9 ± 6.2^{e}
Ultraflo	30.5 ± 0.6^{a}	60.5 ± 0.9^{b}	134.9 ± 5.1^{c}	11.0 ± 0.0^{a}	9.6 ± 0.2^{b}	50.3 ± 1.0^{b}	264.2 ± 5.3^{a}	2.4 ± 0.3^{b}	563.4 ± 9.5^{b}
Pentopan	5.8 ± 0.4^{f}	130.9 ± 7.2^{a}	276.6 ± 4.0^{a}	1.7 ± 0.5^{e}	16.6 ± 0.5^{a}	185.5 ± 6.3^{a}	17.1 ± 0.9^{f}	2.9 ± 0.1^{a}	637.2 ± 18.8^{a}

[a] Values (dry weight basis) with different superscripts within column are different with statistical significance ($p < 0.05$).
[b] PCCA, protocatechuic acid; *p*-OH, *p*-hydroxybenzoic acid; VA, vanillic acid; SyA, syringic acid; CFA, caffeic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; SNA, sinapic acid.
[c] SPC, sum of individual phenolic acids content.

acid, could be effectively extracted in aqueous alcohol solution more than 3 times by the enzymatic treatment. Based on the results of the assays for radical scavenging activity and ferrous reducing activity, the antioxidant activity of the RB extract was significantly improved. The enzymatic procedure used in this study is simple and readily applicable for the production of natural antioxidants from various plant sources including RB.

Acknowledgment

This study was financially supported by the Korean Small and Medium Business Administration (Grants No. S2108305) in 2014.

References

Adom, K.K., Liu, R.H., 2002. Antioxidant activity of grains. J. Agric. Food Chem. 50, 6182–6187.
Alrahmany, R., Avis, T.J., Tsopmo, A., 2013. Treatment of oat bran with carbohydrases increases soluble phenolic acid content and influences antioxidant and antimicrobial activities. Food Res. Int. 52, 568–574.
Alrahmany, R., Tsopmo, A., 2012. Role of carbohydrases on the release of reducing sugar, total phenolics and on antioxidant properties of oat bran. Food Chem. 132, 413–418.
Bagger-Jørgensen, R., Meyer, A.B.S., 2004. Effects of different enzymatic pre-press maceration treatments on the release of phenols into blackcurrant juice. Eur. Food Res. Technol. 219, 620–629.
Balasubashini, M.S., Rukkumani, R., Menon, V.P., 2003. Protective effects of ferulic acid on hyperlipidemic diabetic rats. Acta Diabetol. 40, 118–122.
Benzie, I.F.F., Szeto, Y.T., 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J. Agric. Food Chem. 47, 633–636.
Butsat, S., Siriamornpun, S., 2010. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. Food Chem. 119, 606–613.
Cerda, A., Martínez, M.E., Soto, C., Poirrier, P., Perez-Correa, J.R., Vergara-Salinas, J.R., Zúñiga, M.E., 2013. The enhancement of antioxidant compounds extracted from *Thymus vulgaris* using enzymes and the effect of extracting solvent. Food Chem. 139, 138–143.
Chen, S., Xing, X.-H., Huang, J.-J., Xu, M.-S., 2011. Enzyme-assisted extraction of flavonoids from *Ginkgo biloba* leaves: improvement effect of flavonol transglycosylation catalyzed by *Penicillium decumbens* cellulase. Enzyme Microb. Technol. 48, 100–105.
DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Faulds, C.B., Mandalari, G., LoCurto, R., Bisignano, G., Waldron, K.W., 2004. Arabinoxylan and mono-and dimeric ferulic acid release from brewer's grain and wheat bran by feruloyl esterases and glycosyl hydrolases from *Humicola insolens*. Appl. Microbiol. Biotechnol. 64, 644–650.
Gioia, D.D., Sciubba, L., Leonardo Setti, L., Luziatelli, F., Ruzzi, M., Zanichelli, D., Fava, F., 2007. Production of biovanillin from wheat bran. Enzyme Microb. Technol. 41, 498–505.
Gomez-Garcia, R., Martinez-Avila, G.G., Aguilar, C., 2012. Enzyme-assisted extraction of antioxidative phenolics from grape (*Vitis vinifera* L.) residues. 3 Biotech 2, 297–300.
Hudson, E.A., Dinh, P.A., Kokubun, T., Simmonds, M.S.J., Gescher, A., 2000. Chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol. Biomarkers Prev. 9, 1163–1170.
Jodayree, S., Smith, J.C., Tsopmo, A., 2012. Use of carbohydrase to enhance protein extraction efficiency and antioxidative properties of oat bran protein hydrolysates. Food Res. Int. 46, 69–75.
Jung, E.H., Kim, S.R., Hwang, I.K., Ha, T.Y., 2007. Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-*db*/*db* mice. J. Agric. Food Chem. 55, 9800–9804.
Kapasakalidis, P.G., Rastall, R.A., Gordon, M.H., 2009. Effect of a Cellulase treatment on extraction of antioxidant phenols from black currant (*Ribes nigrum* L.) pomace. J. Agric. Food Chem. 57, 4342–4351.
Kim, Y.J., Kim, D.-O., Chun, O.K., Shin, D.-H., Jung, H., Lee, C.Y., Wilson, D.B., 2005. Phenolic extraction from apple peel by cellulases from *Thermobifida fusca*. J. Agric. Food Chem. 53, 9560–9565.
Li, B.B., Smith, B., Hossain, M.M., 2006. Extraction of phenolics from citrus peels: II. Enzyme-assisted extraction method. Sep. Purif. Technol. 48, 189–196.
Mathew, S., Abraham, T.E., 2006. Bioconversions of ferulic acid, an hydroxycinnamic acid. Crit. Rev. Microbiol. 32, 115–125.
Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.
Moore, J., Cheng, Z., Su, L., Yu, L., 2006. Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. J. Agric. Food Chem. 54, 9032–9045.
Nam, S.H., Choi, S.P., Kang, M.Y., Kozukue, N., Friedman, M., 2005. Antioxidative, antimutagenic, and anticarcinogenic activities of rice bran extracts in chemical and cell assays. J. Agric. Food Chem. 53, 816–822.
Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53, 4290–4302.
Ti, H., Li, Q., Zhang, R., Zhang, M., Deng, Y., Wei, Z., Chi, J., 2014. Free and bound phenolic profiles and antioxidant activity of milled fractions of different *indica* rice varieties cultivated in southern China. Food Chem. 159, 166–174.
Tripathi, U., Rao, S.R., Ravishankar, G.A., 2002. Biotransformation of phenylpropanoid compounds to vanilla flavor metabolites in cultures of *Haematococcus pluvialis*. Process Biochem. 38, 419–426.
Wang, T., Jónsdóttirb, R., Kristinssonb, H.G., Hreggvidssonb, G.O., Jónssonb, J.O., Thorkelssonb, G., Ólafsdóttire, G., 2010. Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmaria palmate*. LWT-Food Sci. Technol. 43, 1387–1393.
Yadav, G., Singh, A., Bhattacharya, P., Yuvraj, J., Banerjee, R., 2013. Comparative analysis of solid-state bioprocessing and enzymatic treatment of finger millet for mobilization of bound phenolics. Bioprocess Biosyst. Eng. 36, 1–7.
Zhou, Z., Robards, K., Helliwell, S., Blanchard, C., 2004. The distribution of phenolic acids in rice. Food Chem. 87, 401–406.