Food Chemistry 221 (2017) 636–643

Contents lists available at ScienceDirect

Food Chemistry

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

Fermentation and complex enzyme hydrolysis enhance total phenolics and antioxidant activity of aqueous solution from rice bran pretreated by steaming with α-amylase

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ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form 17 November 2016
Accepted 22 November 2016
Available online 23 November 2016

Chemical compounds studied in this article:
Ferulic acid (Pubchem CID: 445858)
Protocatechuic acid (Pubchem CID: 72)
Coumaric acid (Pubchem CID: 637542)
Isoferulic acid (Pubchem CID: 736186)
Chlorogenic acid (Pubchem CID: 1794427)
Caffeic acid (Pubchem CID: 689043)
Gallic acid (Pubchem CID: 370)
Syringic acid (Pubchem CID: 10742)
Vanillic acid (Pubchem CID: 8468)
Catechin (Pubchem CID: 9064)
(−)-Epicatechin (PubChem CID: 72276)
Kaempferol (Pubchem CID: 5280863)

Keywords:
Rice bran
Lactic acid bacteria fermentation
Complex enzyme hydrolysis
Soluble conjugate phenolics
Phenolic acids
Flavonoids
Antioxidant activity

ABSTRACT

In this study, rice bran was successively steamed with α-amylase, fermented with lactic acid bacteria, and hydrolyzed with complex enzymes. The changes in phenolic profiles and antioxidant activities of the corresponding aqueous solutions from three stages were investigated. Compared to the first stage, fermentation and complex enzyme hydrolysis significantly increased the total phenolics, total flavonoids, total FRAP and ORAC values by 59.2%, 56.6%, 73.6% and 45.4%, respectively. Twelve individual phenolics present in free or soluble conjugate forms were also analyzed during the processing. Ferulic acid was released in the highest amount among different phenolics followed by protocatechuic acid. Moreover, a major proportion of phenolics existed as soluble conjugates. The results showed that fermentation and complex enzyme hydrolysis enhanced total phenolics and antioxidant activities of aqueous solution from rice bran pretreated by steaming with α-amylase. This research could provide basis for the processing of rice bran beverage rich in phenolics.

1. Introduction

Rice bran is a by-product of rice milling process and is a good source of nutrients rich in dietary fiber, protein, vitamins and phytochemicals (Friedman, 2013). Due to these important nutritional values, there has been increasing interest in utilizing rice bran in processed foods, and it has already been used in foods such as baked products, beverages and meatballs (Hu & Yu, 2015). Some epidemiological studies suggest that intake of rice bran has been associated with the prevention of chronic diseases such as cancer and cardiovascular diseases (Kannan, Hettiarachchy, Johnson, & Nannapaneni, 2008; Okarter & Liu, 2010; Verschoyle et al., 2007). These health benefits have been partly attributed to the unique phytochemical content of rice bran.

Phenolics, as one of the most abundant types of phytochemicals in rice bran, are considered to be a natural antioxidant and exist in free, soluble conjugate, and insoluble bound forms (Adom & Liu, 2002; Wang et al., 2015). Most of researches have focused on free

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http://dx.doi.org/10.1016/j.foodchem.2016.11.126
0308-8146/

and insoluble bound forms of rice phenolics while ignoring soluble conjugate form (Min, Gu, McClung, Bergman, & Chen, 2012; Ti et al., 2014), which could be equally important. For example, like insoluble bound form, soluble conjugate phenolics may also play an essential role in delivering antioxidants to the colon upon release caused by bacterial microbiota (Saura-Calixto, 2011; Zhang et al., 2014). This suggests that the soluble conjugate form is an important source of phenolic compounds in cereals. In barley and some cranberry beans, the content of soluble conjugate phenolics is higher than that of the free form (Chen et al., 2015; Madhujith & Shahidi, 2009). Therefore, it is important to determine the soluble conjugate phenolic content and its antioxidant activity in rice bran to better understand the potential health benefits.

Fermentation is a biotechnological process used to enhance the shelf-life and the nutritional and organoleptic qualities of foods (Hur, Lee, Kim, Choi, & Kim, 2014). Over the past decades, numerous fermentation studies on rice bran have been conducted using lactic acid bacteria with the main purpose of producing lactic acid (Gao, Kaneko, Hirata, Toorisaka, & Hano, 2008; Li, Lu, Yang, Han, & Tan, 2012). A few studies have investigated the effect of fermentation on phenolic content and antioxidant activity of rice bran (Martins et al., 2011). Dordević, Šiler-Marinković, and Dimitrijević-Branković (2010) reported that lactic acid fermentation can increase the phenolic content and antioxidant activity in some cereals, but the efficiency was very low. A recent study using fungus reported that solid state fermentation increased the phenolic content of rice bran (Schmidt, Gonçalves, Prietto, Hackbart, & Furlong, 2014). Unfortunately, the soluble conjugate phenolic content and antioxidant activity were neglected in the above research, and therefore, the total phenolic content was underestimated. In addition to fermentation, enzymatic hydrolysis is often used for cereal processing due to its low cost, simple equipment and environmental compatibility. The cell wall of rice bran is a compact and complex three-dimensional structure and has a strong binding affinity to phenolics. Therefore, it is desirable to further degrade the cell wall of rice bran using complex enzymes to release phenolics. Alrahmany and Tsopmo (2012) reported four different carbohydrase treatments that increased the soluble phenolic content and antioxidant activity of oat bran. To date, the processing of rice bran by a combination of fermentation and enzymatic treatments has not been studied in detail. It is largely unknown how this combination of lactic acid bacteria fermentation and enzymatic treatment may affect the phenolic compositions, including flavonoids, and the antioxidant activity of rice bran.

In the present study, rice bran was successively steamed with α-amylase (liquefaction stage), fermented with lactic acid bacteria (fermentation stage), and hydrolyzed with complex enzymes (complex enzyme hydrolysis stage). The objectives of this study were to: (1) investigate changes in the free and soluble conjugate phytochemical (phenolics and flavonoiods) contents of aqueous solutions from rice bran and their antioxidant activity at different stages; and (2) characterize changes in the compositions and content of individual phenolic compounds in free and soluble conjugate forms in aqueous solutions during the process. The present research could provide guidance for the processing of rice bran beverage rich in phenolics.

2. Materials and methods

2.1. Chemicals and reagents

All reagents, unless otherwise noted, were of analytical grade or above. Analytical grade methanol (MeOH), ethanol, ethyl acetate, hexanes, citric acid, hydrochloric acid (HCl), sodium acetate (NaAc), acetic acid (HAc), potassium chloride (KCl), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), potassium phosphate monobasic (KH_2PO_4) and potassium phosphate dibasic (K_2HPO_4) were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). Folin–Ciocalteu reagent, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,20-azobis(2-amidinopropane)dihydrochloride (ABAP), fluorescein disodium salt and 2,4,6-tripyridyl-s-triazine (TPTZ), were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Individual phenolic standards were obtained from Aladdin Reagents (Shanghai, China). Food grade α-amylase (20,000 uints/g), glucoamylase (100,000 uints/g), acid protease (50,000 uints/g) and acid cellulase (35,000 uints/g) were obtained from Youtell Biochemical Com., Ltd (Shanghai, China). HPLC grade acetonitrile and acetic acid were purchased from Fisher (Suwanee, GA, USA).

2.2. Preparation of starter cultures

The lactic acid bacteria strains, *Lactobacillus acidophilus (GIM1.731) and Lactobacillus plantarum (GIM 1.648)*, were purchased from the Guangdong Microbiology Culture Center within the Guangdong Institute of Microbiology. Lactic acid bacteria cultures were inoculated (1%) in MRS broth (Guangzhou HuanKai Microbiological Technology Co. Ltd., Guangzhou, China) and incubated at 30 °C for 24 h. After three passages, the cells were harvested by centrifugation at 4000 rpm for 15 min, washed twice and resuspended in a sterile saline solution (0.9% NaCl). The two cell suspensions (9 log CFU/mL) were used as a mixed starter culture in a ratio of 1:1 for rice bran fermentation.

2.3. Processing of rice bran

The brown rice was provided by the Rice Research Institute of Guangdong Academy of Agricultural Sciences, China. The rice sample was polished using the rice milling machine to obtain 10% rice bran, which was defatted by supercritical carbon dioxide. The principal constituents of the defatted rice bran were as follows: 9.56% moisture, 41.11% starch, 14.28% protein, 24.65% crude fiber and 9.13% ash. This sample was stored at -20 °C until use.

Based on the preliminary experiments, the rice bran was processed by steaming with α-amylase, fermentating with lactic acid bacteria and hydrolyzing with complex enzyme, in successive order (Wen et al., 2015, 2016). Firstly, 1 mL α-amylase was diluted with 60 mL distilled water and uniformly sprayed onto 40 g rice bran. The rice bran was steamed in an autoclave at 100 °C for 30 min and then inactivated at 121 °C for 10 min (liquefaction stage). The rice bran was cooled to 35.5 °C and was inoculated with 5% lactic acid bacteria for the fermentation of 35 h. The fermented rice bran was mixed with 140 mL distilled water and then pasteurized at 70 °C for 10 min (fermentation stage). Subsequently, the mixture was adjusted to pH 4.1 using citric acid and then incubated with complex enzyme (0.5% glucoamylase, 1.5% acid protease and 1.5% acid cellulase based on the weight of rice bran) for 190 min at 57.5 °C (complex enzyme hydrolysis stage). The mixture was centrifuged at 4000 rpm for 10 min, and the resulting supernatant was mixed with proper organic solvent for the extraction of phenolics, described below. Samples from each of the three stages were collected and stored at −20 °C until analysis. In detail, sample from liquefaction stage was rice bran after steaming at 121 °C, and samples from the other two stages were aqueous solutions from rice bran as treated by pasteurization and complex enzyme hydrolysis, respectively. The processing of rice bran was performed in triplicate.

2.4. Extraction of free phenolics

Free phenolic compounds were extracted based on a previous method with slight modifications (Alrahmany, Avis, & Tsopmo, 2013). Briefly, 5 g rice bran from the liquefaction stage was blended in 50 mL of acidified water (pH 3.0) and partitioned five times with 50 mL of ethyl acetate. 50 mL aqueous solutions from the other two stages were adjusted to pH 3.0 and also partitioned with ethyl acetate for five times. The pooled ethyl acetate fractions were evaporated to dryness at 45 °C. The residue was recovered by adding 10 mL MeOH to yield the free phenolics extract solution, which was then stored at −20 °C until analysis.

2.5. Extraction of soluble conjugate phenolics

The soluble conjugate phenolics were extracted from the water phase after extracting free phenolics according to previous methods (Adom & Liu, 2002; Madhujith & Shahidi, 2009). Briefly, the water phase was hydrolyzed by adding 40 mL of 2 M NaOH at room temperature for 4 h while shaking and under nitrogen gas. The solution was then adjusted to pH 2.0 with 6 M HCl and extracted five times with ethyl acetate as previously described. The residue was recovered by adding 10 mL MeOH to give the soluble conjugate phenolics extract solution, and stored at −20 °C until analysis.

2.6. Determination of total phenolic content

The total phenolic content in both the free and soluble conjugate fractions was determined by the Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, a 125 μL aliquot of the above extract solution was diluted with 0.5 mL distilled water and was subsequently reacted with 125 μL Folin–Ciocalteu reagent for 6 min. Then, 1.25 mL of 7% aqueous sodium carbonate solution was added to the solution to reach a final volume of 3 mL. The reaction solution was incubated in the dark for 90 min, and the absorbance was immediately determined at 760 nm using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan). Gallic acid was used as the standard, and the results were expressed as mg gallic acid equivalents per 100 g dry weight of rice bran (mg GAE/100 g DW).

2.7. Determination of total flavonoid content

The total flavonoid content was determined based on the colorimetric method with slight modifications (Min et al., 2012). A 300 μL aliquot of the above extract solution was diluted with 1.5 mL distilled water and was subsequently reacted with 90 μL 5% $NaNO_2$ solution for 6 min. Then, 180 μL 10% $AlCl_3{\cdot}6H_2O$ solution was added to the solution, and the mixture was incubated for 5 min before adding 0.6 mL 1 M NaOH solution. The mixture was diluted to a final volume of 3 mL with distilled water, and the absorbance was measured at 510 nm using a Shimadzu UV-1800 spectrometer. (+)-catechin was used as the standard, and the results were expressed as mg (+)-catechin equivalents per 100 g dry weight of rice bran (mg CE/100 g DW).

2.8. Determination of phenolic composition

The individual phenolic compounds in the above extracts were quantified using an Agilent 1200 HPLC system (Waldbronn, Germany) equipped with an Agilent 1200 series VWD detector and autosampler. An Agilent Zorbox SB-C_{18} column (250 × 4.6 mm i.d., 5 μm; Palo Alto, CA, USA) was used at a column temperature of 30 °C. The mobile phase consisted of a 0.4% aqueous solution of acetic acid (solution A) and acetonitrile (solution B) with the following gradient program: 0–40 min, solution B 5–25%; 40–45 min, solution B 25–35%; 45–50 min, solution B 35–50%. Other chromatographic conditions included a constant flow rate of 1.0 mL/min, an injection volume of 20 μL, a run time of 50 min, and detection wavelength of 280 nm. Prior to analysis, all samples were filtered through a 0.25-μm membrane filter (Millipore, Billerica, MA, USA). The identification of each peak was based on the retention time and the chromatography of authentic standards. The percent recovery of these phenolics ranged from 90.2–99.1%. The concentrations of each compound were calculated according to a standard curve, and the results were expressed as μg per gram DW of rice bran.

2.9. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the method described by Benzie and Strain (1996). Fresh FRAP working reagent was prepared daily by mixing 25 mL 300 mM acetate buffer (5.1 g $CH_3COONa{\cdot}3H_2O$ in 20 mL CH_3COOH, pH 3.6), 2.5 mL TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 mL of 20 mM $FeCl_3{\cdot}6H_2O$ solution, which was warmed to 37 °C before use. A 30 μL aliquot of the above extracts was mixed with 90 μL of distilled water and was then allowed to react with 900 μL of the working reagent for 30 min in the dark at room temperature. The absorbance was detected at 593 nm using a Shimadzu UV-1800 spectrometer. Trolox was used as the standard, and the FRAP antioxidant activity was expressed as mg Trolox equivalents per 100 g DW of rice bran (mg TE/100 g DW).

2.10. Oxygen radical scavenging capacity (ORAC) assay

The ORAC assay was conducted in black 96-well plates (Corning Scientific, Corning, NY) based on previous methods (Qiu, Liu, & Beta, 2010; Zhang, Zhang, Zhang, & Liu, 2010) with slight modifications. Briefly, the above extract dilutions were prepared with 75 mM phosphate buffer (pH 7.4). The outside wells of the plate were not used. The final reaction mixture contained 20 μL of extract solution or 20 μL of Trolox standard (range: 6.25–50 μM) and 200 μL of fluorescein (final concentration 0.96 μM). The plate was incubated at 37 °C for 20 min in a plate reader. Then, 20 μL of 119 mM ABAP solution was quickly added to each well using a multichannel pipette. The fluorescence intensity was measured using a Fluoroskan Ascent FL plate-reader (Thermo Labsystems, Franklin, MA, USA) at 485 nm for excitation and 538 nm for emission for 35 cycles every 4.5 min. The ORAC value was expressed as micromole Trolox equivalents per gram DW of rice bran (μmol TE/g DW).

2.11. Statistical analyses

Each treatment was repeated in triplicate, and the results were presented on a dry weight basis of original rice bran sample as mean ± standard deviation (SD) of triplicate determinations. Different samples were analyzed using one-way ANOVA followed by the SNK-q test, and the statistical analyses were performed using SPSS13.0 software (SPSS Inc. Chicago, IL, USA). Statistical significance was set at the level of $p < 0.05$.

3. Results

3.1. Total phenolic content

Contents of free, soluble conjugate, and total phenolics of aqueous solutions from rice bran during different stages as well as the contribution of free and soluble conjugate to total phenolics is

shown in Table 1. The total phenolic contents of aqueous solution were significantly different among the three stages ($p < 0.05$).

After steaming with α-amylase, free, soluble conjugate, and total phenolics of aqueous solution were 32.1, 86.4 and 118.5 mg GAE/100 g of DW. Compared to the first stage (liquefaction stage), the lactic acid bacteria fermentation increased the contents of free, soluble conjugate, and total phenolics by 90.7%, 17.2% and 37.1%, respectively, (61.2, 101.3 and 162.5 mg GAE/100 g of DW, respectively). Complex enzyme treatment further increased the contents of free, soluble conjugate, and total phenolics in rice bran extract (85.1, 103.5 and 188.6 mg GAE/100 g of DW, respectively). The free, soluble conjugate, and total phenolics contents were increased by 165.1%, 19.8% and 59.2%, respectively, when compared to the liquefaction stage, and when compared to the fermentation stage, the contents increased by 39.1%, 2.2% and 16.1%, respectively. Moreover, there was no significant difference between the phenolics contents of the soluble conjugate at the fermentation and complex enzyme hydrolysis stages. During the processing, the percentage contribution of the free phenolic fraction to total phenolics increased from 27.1% to 45.1%, while the percentage contribution of the soluble conjugate phenolic fraction to total phenolics decreased from 82.9% to 54.9%. In summary, these results demonstrate that the fermentation and complex enzyme hydrolysis together significantly increased the free, soluble conjugate and total phenolic contents in rice bran extract.

3.2. Total flavonoid content

Contents of free, soluble conjugate, and total flavonoids of aqueous solutions during different stages as well as the contributions of free and soluble conjugate to total flavonoids are shown in Table 2. Total flavonoid contents of aqueous solutions were significantly different among the three stages ($p < 0.05$).

After liquefaction treatment, contents of free, soluble conjugate, and total flavonoids of aqueous solution were 5.5, 26.3 and 31.8 mg CE/100 g of DW. Compared to the liquefaction stage, fermentation further increased the content of free, soluble conjugate, and total flavonoids of aqueous solution by 118.2%, 21.3% and 38.1%, respectively (12.0, 31.9 and 43.9 mg GAE/100 g of DW, respectively). Complex enzyme hydrolysis significantly increased the contents of free, soluble conjugate, and total flavonoids of aqueous solution by 201.8%, 26.2% and 56.6%, respectively (16.6, 33.2 and 49.8 mg CE/100 g of DW, respectively) when compared to those of the liquefaction stage. During the processing, the percentage contribution of the free flavonoid fraction to total flavonoids increased from 17.3% to 33.3%, while the percentage of the soluble conjugate flavonoid fraction to total flavonoids decreased from 82.7% to 66.7%.

Table 1
The contents of free, soluble conjugate and total phenolics of aqueous solutions from rice bran during different stages and the percentage contributions of free and soluble conjugate fractions to the total phenolics.

Stage	Phenolics (mg GAE/100 g DW)		
	Free	Soluble conjugate	Total
L	32.1 + 1.7a[a](27.1) [b]	86.4 + 2.5a(82.9)	118.5 + 2.0a
L + F	61.2 + 1.4b(37.7)	101.3 + 3.3b(62.3)	162.5 + 1.9b
L + F + E	85.1 + 3.3c(45.1)	103.5 + 3.5b(54.9)	188.6 + 3.4c

L:liquefaction (steaming with α-amylase); F: lactic acid bacteria fermentation; E: complex enzyme hydrolysis; GAE: gallic acid equivalents.
[a] Values with different letters in each column are significantly different during different stages($p < 0.05$).
[b] Values in parentheses indicate percentage contribution to the total content.

Table 2
The contents of free, soluble conjugate and total flavonoids of aqueous solutions from rice bran during different stages and the percentage contributions of free and soluble conjugate fractions to the total flavonoids.

Stage	Flavonoids (mg CE/100 g DW)		
	Free	Soluble conjugate	Total
L	5.5 + 1.7a[a](17.3)[b]	26.3 + 1.2a(82.7)	31.8 + 1.4a
L + F	12.0 + 1.4b(27.3)	31.9 + 1.5b(72.7)	43.9 + 1.4b
L + F + E	16.6 + 2.2c(33.3)	33.2 + 2.1b(66.7)	49.8 + 2.2c

L: liquefaction (steaming with α-amylase); F: lactic acid bacteria fermentation; E: complex enzyme hydrolysis; CE: (+)-catechin equivalents.
[a] Values with different letters in each column are significantly different during different stages($p < 0.05$).
[b] Values in parentheses indicate percentage contribution to the total content.

3.3. Phenolic composition

The 12 phenolic compounds in free and soluble conjugate fractions of aqueous solutions from rice bran were analyzed during different stages. The 12 compounds were ferulic acid, protocatechuic acid, chlorogenic acid, caffeic acid, isoferulic acid, coumaric acid, gallic acid, syringic acid, vanillic acid, (−)-epicatechin, catechin and kaempferol. The contents of 12 individual phenolics of aqueous solutions and the percentage contribution of free and soluble conjugate fractions to the total content are shown in Table 3. According to HPLC analyses, the compositions of free and soluble conjugate phenolics of aqueous solutions were similar during different stages, while their contents were significantly different ($p < 0.05$). Most of the measured phenolics were present in both free and soluble conjugate forms in aqueous solutions, with the exception that kaempferol was present only in free form. Coumaric acid and gallic acid were mainly present in free form, while the other individual phenolics were mainly present in soluble conjugate form during the processing. Vanillic acid was mainly present in free form in the liquefaction and complex enzyme hydrolysis stages, but in the fermentation stage, was mainly in the soluble conjugate form.

Generally, the total content of each phenolic compound in aqueous solutions from rice bran significantly increased during the processing ($p < 0.05$). Exceptions included isoferulic acid and coumaric acid, which significantly decreased by 30.7% and 40.8%, respectively, and syringic acid and vanillic acid, which did not significantly change ($p > 0.05$). The increases in the total amount of each phenolic compound were as follows: ferulic acid, 177.1%; protocatechuic acid, 101.0%; chlorogenic acid, 83.5%; caffeic acid, 229.3%; gallic acid, 348.1%; catechin, 107.3%; and (−)-epicatechin, 70.4%. The concentrations of free kaempferol, which was not detected in the liquefaction stage, were 6.5 and 6.0 μg/g in the stages of fermentation and complex enzyme hydrolysis, respectively.

Compared to the liquefaction stage, fermentation significantly increased the content of free protocatechuic acid, chlorogenic acid, gallic acid, catechin, (−)-epicatechin and kaempferol, and decreased the content of free ferulic acid, isoferulic acid, coumaric acid, syringic acid and vanillic acid. The soluble conjugate phenolics in aqueous solutions were also analyzed. Fermentation significantly increased the content of soluble conjugate ferulic acid, protocatechuic acid, chlorogenic acid, caffeic acid, coumaric acid, gallic acid, syringic acid, vanillic acid, catechin and (−)-epicatechin and only decreased the content of soluble conjugate isoferulic acid.

Similarly, compared to the fermentation stage, complex enzyme hydrolysis further increased the content of each free phenolic compound, except gallic acid and kaempfrol, which did not significantly change. Alternatively, complex enzyme hydrolysis

Table 3
Changes of individual phenolics of aqueous solutions from rice bran during different stages and the percentage contribution of free and soluble conjugate fractions to the total content.

Phenolics	Stage	Free (μg/g)	Soluble conjugate(μg/g)	Total (μg/g)
Ferulic acid	L	6.4 ± 0.6a[a](16.1)[b]	33.4 ± 6.7a(83.9)	39.8 ± 3.5a
	L + F	nd	46.7 ± 0.9b⟨1 0 0⟩	46.7 ± 0.9b
	L + F + E	39.4 ± 4.4b(35.7)	70.9 ± 2.3c(64.3)	110.3 ± 3.2c
Protocatechuic acid	L	3.6 ± 0.3a(18.8)	15.5 ± 0.5a(81.2)	19.1 ± 0.4a
	L + F	4.5 ± 0.4b(15.6)	24.4 ± 0.2b(84.4)	28.9 ± 0.3b
	L + F + E	8.9 ± 1.9c(23.2)	29.5 ± 3.1c(76.8)	38.4 ± 2.8c
Isoferulic acid	L	2.8 ± 0.7a(20.0)	11.2 ± 2.0c(80.0)	14.0 ± 1.2c
	L + F	nd	8.0 ± 0.2b⟨1 0 0⟩	8.0 ± 0.2 a
	L + F + E	2.8 ± 0.5a(28.9)	6.9 ± 0.6a(71.1)	9.7 ± 0.6 b
Chlorogenic acid	L	2.5 ± 0.3a(24.3)	7.8 ± 0.1a(75.7)	10.3 ± 0.2a
	L + F	4.3 ± 0.2b(23.9)	13.7 ± 0.3b (76.1)	18.0 ± 0.3b
	L + F + E	5.3 ± 0.6c(28.0)	13.6 ± 0.6b(72.0)	18.9 ± 0.6b
Caffeic acid	L	nd	4.1 ± 0.2a⟨1 0 0⟩	4.1 ± 0.2a
	L + F	nd	10.5 ± 0.2b⟨1 0 0⟩	10.5 ± 0.2b
	L + F + E	1.2 ± 0.1(8.9)	12.3 ± 1.4b(91.1)	13.5 ± 0.8c
Coumaric acid	L	13.3 ± 0.6c(87.5)	1.9 ± 0.1a(12.5)	15.2 ± 0.3c
	L + F	3.3 ± 0.3a(58.9)	2.3 ± 0.2b(41.1)	5.6 ± 0.3a
	L + F + E	5.9 ± 0.9b(65.6)	3.1 ± 0.1c(34.4)	9.0 ± 0.3b
Gallic acid	L	4.6 ± 0.3a(85.2)	0.8 ± 0.3a(14.8)	5.4 ± 0.3a
	L + F	22.3 ± 2.4b(91.8)	2.0 ± 0.3b(8.2)	24.3 ± 0.6b
	L + F + E	21.7 ± 1.0b(89.7)	2.5 ± 0.5b(10.3)	24.2 ± 0.7b
Syringic acid	L	3.8 ± 0.2c(45.2)	4.6 ± 0.1a(54.8)	8.4 ± 0.2b
	L + F	1.4 ± 0.1a(20.9)	5.3 ± 0.1b(79.1)	6.7 ± 0.2a
	L + F + E	2.8 ± 0.3b(34.1)	5.4 ± 0.1b(65.9)	8.2 ± 0.2b
Vanillic acid	L	5.2 ± 0.8b(57.1)	3.9 ± 0.2a(42.9)	9.1 ± 0.6b
	L + F	3.6 ± 0.1a(44.4)	4.5 ± 0.2b(55.6)	8.1 ± 0.2a
	L + F + E	4.9 ± 0.4b(57.0)	4.7 ± 0.2b(43.0)	8.6 ± 0.3b
Catechin	L	2.3 ± 0.2a(21.1)	8.6 ± 0.1a(78.9)	10.9 ± 0.2a
	L + F	9.2 ± 0.4b(41.4)	13.0 ± 0.7b(58.6)	22.2 ± 0.5b
	L + F + E	9.9 ± 0.3c(43.8)	12.7 ± 1.8b(56.2)	22.6 ± 1.1b
(−)-Epicatechin	L	0.7 ± 0.2a(4.9)	13.5 ± 0.8a(95.1)	14.2 ± 0.6a
	L + F	3.5 ± 0.1b(16.8)	17.3 ± 0.8b(83.2)	20.8 ± 0.5b
	L + F + E	7.2 ± 0.7c(29.8)	17.0 ± 0.4b(70.2)	24.2 ± 0.6c
Kaempferol	L	nd	nd	nd
	L + F	6.5 ± 0.1a⟨1 0 0⟩	nd	6.5 ± 0.1a
	L + F + E	6.0 ± 0.5a⟨1 0 0⟩	nd	6.0 ± 0.5a

L: liquefaction (steaming with α-amylase); F: lactic acid bacteria fermentation; E: complex enzyme hydrolysis.
[a] Values with different letters in each column are significantly during different stages($p < 0.05$).
[b] Values in parentheses indicate percentage contribution to the total content.

significantly increased the content of soluble conjugate ferulic acid, protocatechuic acid, coumaric acid and caffeic acid, decreased the content of soluble conjugate isoferulic acid, and had no significant influence on the content of soluble conjugates chlorogenic acid, caffeic acid, gallic acid, syringic acid, vanillic acid, catechin and (−)-epicatechin. Interestingly, the content of free caffeic acid, which was not detected in the stages of liquefaction and fermentation, was 1.2 μg/g after complex enzyme hydrolysis.

3.4. Antioxidant activity by FRAP assay

The contents of free, soluble conjugate, and total antioxidant activities of aqueous solutions as well as the contributions of free and soluble conjugate fractions to the total antioxidant activities, expressed as FRAP values, during different processing stages are shown in Table 4. The total FRAP of aqueous solutions was significantly different among three stages ($p < 0.05$).

After liquefaction treatment, the free, soluble conjugate, and total FRAP values of aqueous solution were 44.3, 169.5 and 213.8 mg TE/100 g DW, respectively. Compared to the liquefaction stage, lactic acid bacteria fermentation significantly increased the free, soluble conjugate, and total FRAP of aqueous solution by 176.1%, 27.6% and 58.3%, respectively (122.3, 216.2 and 338.5 mg TE/100 g DW, respectively). Complex enzyme hydrolysis further increased the free, soluble conjugate, and total FRAP of aqueous solution by 269.3%, 22.4%, and 73.6%, respectively (163.6, 207.5

Table 4
Antioxidant activity of the free, soluble conjugate and total fractions of aqueous solutions from rice bran during different stages and the percentage contributions of free and soluble conjugate fractions to the total antioxidant activity.

Stage	Antioxidant Activity		
	Free	Soluble conjugate	Total
FRAP value (mg TE/100 g DW)			
L	44.3 + 5.5a[a] (20.7) [b]	169.5 + 10.4a(79.3)	213.8 + 7.9a
L + F	122.3 + 6.4b(36.1)	216.2 + 6.7b(63.9)	338.5 + 6.6b
L + F + E	163.6 + 16.3c(44.1)	207.5 + 21.7b(55.9)	371.1 + 18.9c
ORAC value (μmol TE/g DW)			
L	2.6 + 0.2a(16.0)	13.7 + 0.4a(84.0)	16.3 + 0.3a
L + F	5.3 + 0.1b(25.0)	15.9 + 0.5b(75.0)	21.2 + 0.4b
L + F + E	7.5 + 0.3c(31.6)	16.3 + 0.5b(68.4)	23.7 + 0.5c

L: liquefaction (steaming with α-amylase); F: lactic acid bacteria fermentation; E: complex enzyme hydrolysis; TE: Trolox equivalents.
[a] Values with different letters in each column are significantly different during different stages($p < 0.05$).
[b] Values in parentheses indicate percentage contribution to the total antioxidant activity.

and 371.1 mg TE/100 g DW, respectively) when compared to values from the liquefaction stage, and when compared to the values of fermentation stage, the values increased by 33.8%, −4.0% ('−'means decrease) and 9.6%, respectively. During the processing, the percentage contribution of free FRAP fraction to total FRAP increased from 20.7% to 44.1%, while the percentage contribution of the soluble conjugate FRAP fraction to total FRAP decreased from 79.3% to 55.9%.

3.5. Antioxidant activity by ORAC assay

The contents of free and soluble conjugate, and the total antioxidant activities of aqueous solutions, as well as the contributions of free and soluble conjugate fractions to the total antioxidant activities, expressed as ORAC values, during different stages are shown in Table 4. The total ORAC of aqueous solutions were significantly different among three stages ($p < 0.05$).

After liquefaction treatment, the free, soluble conjugate, and total ORAC of aqueous solution were 2.6, 13.7 and 16.3 μmol TE/g DW. Fermentation significantly increased the free, soluble conjugate, and total ORAC of aqueous solution by 103.8%, 2.5% and 30.1%, respectively (5.3, 15.9 and 21.2 μmol TE/g DW, respectively), when compared to the values of the liquefaction stage. Complex enzyme hydrolysis further increased the free, soluble conjugate, and total ORAC of aqueous solutions by 41.5%, 2.5% and 11.8%, respectively (7.5, 16.3 and 23.7 μmol TE/g DW, respectively) when compared to values of the fermentation stage. Moreover, there was no significant difference in the soluble conjugate antioxidant activity between the fermentation and complex enzyme hydrolysis stages. During the processing, the percentage contribution of free ORAC fraction to the total increased from 16.0% to 31.6%, while the percentage of soluble conjugate ORAC fraction to the total decreased from 84.0% to 68.4%.

4. Discussion

4.1. Effect on the total soluble phenolic content

Starch is a main component of rice bran. Degrading the starch structure in a semi-solid condition is a key to the utilization of rice bran. Myat and Ryu (2014) found that extrusion with α-amylase at 115 °C and 135 °C effectively gelatinized and degraded native corn starch. In this research, steaming with thermostable α-amylase was used to gelatinize and liquefy the starch in rice bran. This processing can provide available sugars for lactic acid bacteria growth in the following fermentation stage. Our results indicate that fermentation increased the total phenolic content in the aqueous solution from rice bran by 37.1%, compared to liquefaction stage. This is mainly because lactic acid bacteria utilize polysaccharides and produce phenolic esterase and carbohydrase, which hydrolyze the ester bond between phenolics and cell wall components (Donaghy, Kelly, & McKay, 1998; Wang, Geng, Egashira, & Sanada, 2004). However, Webber, Hettiarachchy, Li, Horax, and Theivendran (2014) reported that fermentation with *Lactobacillus acidophilus* failed to release phenolics from defatted rice bran because the rice bran had not been pretreated by gelatinization and liquefaction. These data suggest that gelatinization and liquefaction are important to improve the fermentation efficiency of lactic acid bacteria in rice bran. This notion was supported by Dordević et al. (2010), who found that lactic acid bacteria fermentation enhanced the total phenolic content of 4 different cereals gelatinized in an autoclave. Furthermore, fermentation by complex strains was more effective than use of a single strain, which is consistent with a previous study. Razak, Rashid, Jamaluddin, Sharifudin, and Long (2015) reported that amplification of phenolic compound extractions derived from rice bran water and methanol extracts was achieved when using a combination of *Rhizopus oligosporus* and *Monascus purpureus*.

Until this study, few studies reported the effect of fermentation on soluble conjugate phenolics in cereal. The results from the present study show that fermentation significantly increased the content of soluble conjugate phenolics of aqueous solution from rice bran, and the conjugate phenolics is a predominant contributor to the total phenolic content. These compounds are considered to be bound to soluble oligosaccharides and peptides through hydrophobic and covalent ester and ether bonds, and can be released upon alkaline hydrolysis (Chen et al., 2015; Saura-Calixto, 2011). Schmidt et al. (2014) reported that fermentation with *Rizhopus* oryzae increased the free phenolic content in rice bran; however, the soluble conjugate phenolics were not studied in this research. Therefore, it is important to perform a comprehensive evaluation of the phenolic profile in cereals, including an analysis of the soluble conjugate phenolics.

In order to further release phenolics, complex enzyme hydrolysis was used after fermentation. The results from this study indicated that complex enzyme hydrolysis significantly increased the free phenolic content and had no significant effect on the conjugate phenolic content when compared to that of the fermentation stage. Large amounts of insoluble bound phenolics are covalently conjugated to cell wall components, such as polysaccharides, cellulose, pectin and lignin, through ester bonds (Chen et al., 2015). Together, glucoamylase, cellulase and protease hydrolyze polysaccharides, protein and cellulose, decrease the molecular mass and size of cell wall components, break the cross-linking machinery within the cell wall structure, and thus, enhance the release of phenolics from rice bran. Previous studies have shown the positive effect of enzyme hydrolysis on the release of phenolics. Ti et al. (2015) reported that digestion with pepsin and pancreatin significantly increased the total free phenolics in brown rice after cooking (Ti, Zhang, Li, Wei, & Zhang, 2015). Alrahmany and Tsopmo (2012) also reported that 4 different carbohydrases increased the total phenolic content in oat bran. This study contradicted the report of Wanyo, Meeso, and Siriamornpun (2014) in which single cellulase failed to increase the phenolics in rice bran. This low release efficiency was mainly due to the fact that the rice bran was not pretreated with gelatinization and liquefaction.

4.2. Effect on the phenolic compositions

We investigated the change of individual phenolics of aqueous solutions throughout the processing. Nine phenolic acids (ferulic, protocatechuic, isoferulic, chlorogenic, caffeic, coumaric, gallic, vanillic and syringic acids) and three flavonoids (catechin, (-)-epicatechin and kaemperol) were studied because they are main phenolic compounds in rice bran (Liu et al., 2015; Ti et al., 2015). Generally, fermentation and complex enzyme treatment, when used together, increased the total content of each phenolic compound, except isoferulic and coumaric acids (Table 3). After complex enzyme treatment, ferulic acid was the most abundant individual phenolic in aqueous solution, followed by protocatechuic acid, while syringic acid was the least abundant. It is easily deduced that the increased amount of phenolic compounds mainly arise from the release of insoluble bound phenolics, such as ferulic acid and protocatechuic acid. It is also possible that some of the free phenolics were generated by hydrolysis of the soluble conjugate phenolics.

Our results demonstrated that a large amount of phenolic compounds (including ferulic acid, chlorogenic acid, caffeic acid, gallic acid catechin, (−)-epicatechin and kaempferol) was released in free or soluble conjugate forms during the fermentation stage. This was consistent with previous reports in which fermentation increased

some free phenolic acids in rice bran (Razak et al., 2015; Schmidt et al., 2014). Compared to the fermentation stage, complex enzyme hydrolysis stage released more ferulic acid. As also described by Yu, Maenz, Mckinnon, Racz, and Christensen (2002), *Aspergillus* ferulic acid esterase and *Trichoderma* xylanase acted synergistically to release ferulic acid from oat hulls. Alrahmany et al. (2013) found that cellulase or α-amylase treatments released phenolic acids in both free and soluble conjugate forms, including ferulic, coumaric, caffeic and vanillic acids in oat bran. Unlike the releasing of ferulic acid in the present study, protocatechuic acid was released in almost comparable level in the stages of fermentation and complex enzyme hydrolysis. In general, the changes in the contents of phenolic compounds were different during the processing. The amount of phenolics generated depends on fermentation conditions, enzymatic treatments and the type of bonds between the phenolics and cell wall components.

4.3. Effect on the antioxidant activity

Phenolics are an important contributor to the antioxidant activity of plant food. A few studies have investigated the changes in the free and soluble conjugate antioxidant activities in rice bran during processing. Two methods were used to determine the antioxidant activity of aqueous solutions from rice bran in this research. The FRAP assay is based on electron transfer (ET) and the ORAC assay is based on hydrogen atom transfer (HAT) (Prior, Wu, & Schaich, 2005). Our results showed that lactic acid bacteria fermentation significantly increased the free and soluble conjugate antioxidant activities of aqueous solutions, and these results were consistent with the changes in the contents of free and soluble conjugate phenolics in the fermentation stage. Generally, the trends in the changes of antioxidant activities measured by FRAP and ORAC assays were consistent. The increase in the antioxidant activity was mainly due to the fermentation stage in which antioxidative phenolics were released and the bioavailability of free hydroxyl groups increased. This result was consistent with previous studies. Dordević et al. (2010) reported that fermentation with *Lactobacillus rhamnosus* increased the free antioxidant activity of cereals measured by FRAP method. Another report also found that fermentation with *Rhizopus oligosporus* and *Monascus purpureus* enhanced the free antioxidant activity measured by FRAP and DPPH assays (Razak et al., 2015). However, it is difficult to compare the antioxidant activities from our study to those reported by other researchers due to the different analytical and quantification methods used.

Moreover, compared to fermentation stage, complex enzyme hydrolysis increased the free antioxidant activity and had no significant effect on the soluble conjugate antioxidant activity of aqueous solutions. A similar phenomenon was observed in our previous report, which showed that digestion with complex enzymes significantly increased the total ORAC values in brown rice after cooking (Ti et al., 2015). Alrahmany and Tsopmo (2012) also reported that 4 different carbohydrases increased the total ORAC values in oat bran. The enhancement of antioxidant activity was closely related to the increase of phenolic compounds in rice bran (Min et al., 2012; Zhang et al., 2010). Another explanation is that enzyme hydrolysis modifies the galloylated form of phenolics to the higher antioxidant activity in the form of phenolic acids, especially gallic acid (Xu et al., 2014). It should be noted that peptides and polysaccharides generated from the hydrolysis of rice bran possibly contributed to the antioxidant activity (Jodayree, Smith, & Tsopmo, 2012). The results of this study show that the soluble conjugate fraction was the primary contributor to the antioxidant activity of aqueous solutions from rice bran at all the three stages. Therefore, it is necessary to analyze the soluble conjugate form of antioxidant activity for a comprehensive evaluation of antioxidant activity of cereal.

5. Conclusion

In the present study, rice bran was successively steamed with α-amylase, fermented with lactic acid bacteria, and hydrolyzed with complex enzymes. This is a food processing procedure, which can be used for the production of rice bran beverage. We concerned the changes in the phenolic profile and antioxidant activity of aqueous solutions from rice bran during the processing. Our results showed that fermentation and complex enzyme hydrolysis enhanced the total phenolics and flavonoids of aqueous solutions from rice bran pretreated by α-amylase. Moreover, a major proportion of phenolics and flavonoids of aqueous solutions existed in soluble conjugate form, which was often neglected in previous studies. Furthermore, a change in the profile of phenolic compounds was observed, with ferulic acid having the largest increase. The antioxidant activity of aqueous solutions also increased after processing. This study provides useful information for processing rice bran into an ingredient that is rich in phenolics and flavonoids for producing functional foods with increased antioxidant activities.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements

This work was supported by the National Key Research Project of China (No. 2016YFD0400702), the National Nature Science Foundation of China (Grant No. 31501478), the Group Program of Natural Science Foundation of Guangdong Province (2016A030312001), the Special Fund for Agro-scientific Research in the Public Interest (Grant No. 201303071 & 201403063), the Guangdong Provincial Science and Technology Project (Grant No. 2016B070701012 & 2016B020203004), and the Pearl River S&T Nova Program of Guangzhou (Grant No. 201506010028).

References

Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*, 6182–6187.
Alrahmany, R., Avis, T. J., & Tsopmo, A. (2013). Treatment of oat bran with carbohydrases increases soluble phenolic acid content influences antioxidant and antimicrobial activities. *Food Research International, 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 Chemistry, 132*, 413–418.
Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Analytical Biochemistry, 239*, 70–76.
Chen, P. X., Tang, Y., Marcone, M. F., Pauls, P. K., Zhang, B., Liu, R. H., et al. (2015). Characterization of free, conjugated and bound phenolics and lipophilic antioxidants in regular- and non-darkening cranberrybeans (*Phaseolus vulgaris* L.). *Food Chemistry, 185*, 298–308.
Donaghy, J., Kelly, P. F., & McKay, A. M. (1998). Detection of ferulic acid esterase production by Bacillus spp. and lactobacilli. *Applied Microbiology and Biotechnology, 50*, 257–260.
Dordević, T. M., Šiler-Marinković, S. S., & Dimitrijević-Branković, S. I. (2010). Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chemistry, 119*, 957–963.
Friedman, M. (2013). Rice brans, rice bran oils, and rice hulls: Composition, food and industrial uses, and bioactivities in humans, animals, and cells. *Journal of Agricultural and Food Chemistry, 61*, 10626–10641.
Gao, M. T., Kaneko, M., Hirata, M., Toorisaka, E., & Hano, T. (2008). Utilization of rice bran as nutrient source for fermentative lactic acid production. *Bioresource Technology, 99*, 3659–3664.
Hu, G. H., & Yu, W. J. (2015). Effect of hemicellulose from rice bran on low fat meatballs chemical and functional properties. *Food Chemistry, 186*, 239–243.
Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I., & Kim, G. B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry, 160*, 346–356.

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 Research International, 46*, 69–75.
Li, Z., Lu, J., Yang, Z., Han, L., & Tan, T. (2012). Utilization of white rice bran for production of L-lactic acid. *Biomass and Bioenergy, 39*, 53–58.
Liu, L., Guo, J. J., Zhang, R. F., Wei, Z. C., Deng, Y. Y., Guo, J. X., et al. (2015). Effect of degree of milling on phenolic profiles and cellular antioxidant activity of whole brown rice. *Food Chemistry, 100*, 1496–1503.
Madhujith, T., & Shahidi, F. (2009). Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chemistry, 117*, 615–620.
Martins, S., Mussatto, S. I., Martínez-Avila, G., Montañez-Saenz, J., Aguilar, C. N., & Teixeira, J. A. (2011). Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. *Biotechnology Advances, 29*, 365–373.
Min, B., Gu, L., McClung, A. M., Bergman, C. J., & Chen, M. H. (2012). Free and bound total phenolic concentrations, antioxidant capacities, and profiles of different bran color. *Food Chemistry, 133*, 715–722.
Myat, L., & Ryu, G. H. (2014). Effect of thermostable α-amylase injection on mechanical and physiochemical properties for saccharification of extruded corn starch. *Journal of the Science of Food and Agriculture, 94*, 288–295.
Kannan, A., Hettiarachchy, N., Johnson, M. G., & Nannapaneni, R. (2008). Human colon and liver cancer cell proliferation inhibition by peptide hydrolysates derived from heat-stabilized defatted rice bran. *Journal of Agriculture and Food Chemistry, 56*, 11643–11647.
Okarter, O., & Liu, R. H. (2010). Health benefits of whole grain phytochemicals. *Critical Reviews in Food Science and Nutrition, 50*, 193–208.
Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.
Qiu, Y., Liu, Q., & Beta, T. (2010). Antioxidant properties of commercial wild rice and analysis of soluble and insoluble phenolic acids. *Food Chemistry, 121*, 140–147.
Saura-Calixto, F. (2011). Dietary fiber as a carrier of dietary antioxidants: An essential physiological function. *Journal of Agriculture and Food Chemistry, 59*, 43–49.
Razak, D. L. A., Rashid, N. Y. A., Jamaluddin, A., Sharifudin, S. A., & Long, K. (2015). Enhancement of phenolic acid content and antioxidant activity of rice bran fermented with *Rhizopus oligosporus* and *Monascus purpureus*. *Biocatalysis and Agricultural Biotechnology, 4*, 33–38.
Schmidt, C. G., Gonçalves, L. M., Prietto, L., Hackbart, H. S., & Furlong, E. B. (2014). Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus *Rizhopus* oryzae. *Food Chemistry, 146*, 371–377.
Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.
Ti, H. H., Guo, J. J., Zhang, R. F., We, Z. C., Liu, L., Bai, Y. J., et al. (2015). Phenolic profiles and antioxidant activity in four tissue fractions of whole brown rice. *RSC Advances, 5*, 101507–101518.
Ti, H. H., Li, Q., Zhang, R. F., Zhang, M. W., Deng, Y. Y., Wei, Z. C., et al. (2014). Free and bound phenolic profiles and antioxidant activity of milled fractions of different *indica* rice varieties cultivated in southern China. *Food Chemistry, 159*, 166–174.
Ti, H. H., Zhang, R. F., Li, Q., Wei, Z. C., & Zhang, M. W. (2015). Effects of cooking and in vitro digestion of rice on phenolic profiles and antioxidant activity. *Food Research International, 76*, 813–820.
Verschoyle, R. D., Greaves, P., Cai, H., Edwards, R. E., Steward, W. P., & Gescher, A. J. (2007). Evaluation of the cancer chemopreventive efficacy of rice bran in genetic mouse models breast, prostate and intestinal carcinogenesis. *British Journal of Cancer, 96*, 248–254.
Wang, X. K., Geng, X., Egashira, Y., & Sanada, H. (2004). Purification and characterization of a feruloyl esterase from the intestinal bacterium Lactobacillus acidophilus. *Applied and Environmental Microbiology, 70*, 2367–2372.
Wang, W., Guo, J., Zhang, J. N., Peng, J., Liu, T. X., & Xin, Z. H. (2015). Isolation, identification and antioxidant activity of bound phenolic compounds present in rice bran. *Food Chemistry, 171*, 40–49.
Wanyo, P., Meeso, N., & Siriamornpun, S. (2014). Effects of different treatments on the antioxidant properties and phenolic compounds of rice bran and rice husk. *Food Chemistry, 157*, 457–463.
Webber, D. M., Hettiarachchy, N. S., Li, R. Q., Horax, R., & Theivendran, S. (2014). Phenolic profile and antioxidant activity of extracts prepared from fermented heat-stabilized defatted rice bran. *Journal of Food Science, 11*, 2383–2391.
Wen, W., Liu, L., Zhang, M. W., Wei, Z. C., Tang, X. J., & Ma, Y. X. (2015). The optimal composite enzymatic hydrolysis process on defatted rice bran and its nutritional evaluation. *Scientia Agricultural Sinica, 48*, 1597–1608.
Wen, W., Zhang, M. W., Liu, L., Zhang, R. F., Wei, Z. C., Zhang, Y., ... Zhang, H. (2016). The effect of lactic bacteria fermentation on the content of sugars and polyphenols of defatted rice bran. *Modern Food Science and Technology, 32*, 137–141.
Xu, C. M., Yagiz, Y., Borejsza-Wysocki, W., Lu, J., Gu, L. W., Ramírez-Rodrigues, M. M., et al. (2014). Enzyme release of phenolics from muscadine grape (*Vitis rotundifolia* Michx.) skins and seeds. *Food Chemistry, 157*, 20–29.
Yu, P. Q., Maenz, D. D., Mckinnon, J. J., Racz, V. J., & Christensen, D. A. (2002). Release of ferulic acid from oat hulls by *Aspergillus* ferulic acid esterase and *Trichoderma* xylanase. *Journal of Agricultural and Food Chemistry, 50*, 1625–1630.
Zhang, C., Monk, J. M., Lu, J. T., Zarepoor, L., Wu, W., Liu, R., et al. (2014). Cooked navy and black bean diets improve biomarkers of colon health and reduce inflammation during colitis. *British Journal of Nutrition, 111*, 1549–1563.
Zhang, M. W., Zhang, R. F., Zhang, F. X., & Liu, R. H. (2010). Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *Journal of Agricultural and Food Chemistry, 58*, 7580–7587.