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Complex enzyme hydrolysis releases antioxidative phenolics from rice bran

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Caffeic acid (Pubchem CID: 689043)
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ABSTRACT

In this study, phenolic profiles and antioxidant activity of rice bran were analyzed following successive treatment by gelatinization, liquefaction and complex enzyme hydrolysis. Compared with gelatinization alone, liquefaction slightly increased the total amount of phenolics and antioxidant activity as measured by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays. Complex enzyme hydrolysis significantly increased the total phenolics, flavonoids, FRAP and ORAC by 46.24%, 79.13%, 159.14% and 41.98%, respectively, compared to gelatinization alone. Furthermore, ten individual phenolics present in free or soluble conjugate forms were also analyzed following enzymatic processing. Ferulic acid experienced the largest release, followed by protocatechuic acid and then quercetin. Interestingly, a major proportion of phenolics existed as soluble conjugates, rather than free form. Overall, complex enzyme hydrolysis releases phenolics, thus increasing the antioxidant activity of rice bran extract. This study provides useful information for processing rice bran into functional beverage rich in phenolics.

1. Introduction

Rice bran is one of the major by-products of rice milling, making up about 8% of rice bran, and is composed of pericarp, aleurone, and subaleurone, as well as smaller amounts of germ and endosperm (Friedman, 2013). It has gained increasing attention worldwide due to its many beneficial nutritional and biological effects. However, it is presently underutilized due to its poor flavor and solubility, and its primary current use is in the production of fertilizer and animal feed. Therefore, how to best utilize rice bran has become an intense focus of research.

One benefit of rice bran is that it is a source of bioactive phenolics. These phenolic compounds have potent antioxidant and free radical scavenging properties, which prevent chronic diseases, such as cancer, diabetes, obesity and cardiovascular diseases (Lai, Chen, Chen, Chang, & Cheng, 2012; Okarter & Liu, 2010; Verschoyle et al., 2007). Our recently published study reported that the total amounts of phenolics and flavonoids were 13.1 and 10.4 times higher, respectively, in the rice bran than in the endosperm (Ti et al., 2014). Phenolics are found in multiple forms in cereal, including soluble free, soluble conjugates and insoluble bound forms (Adom & Liu, 2002; Wang et al., 2015). Of these forms, the soluble conjugate phenolics in cereals have not received as much attention as free and bound forms. Madhujith and Shahidi (2009) observed that there are higher amounts of soluble conjugate than free phenolics in barley. Furthermore, soluble conjugate phenolics, once released from ingested food by bacteria in the microbiota, may play an essential role in delivering antioxidants to the colon

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in a manner similar to bound phenolics (Saura-Calixto, 2011; Zhang et al., 2014). These reports suggest that the soluble conjugate form is an important source of phenolic compounds in cereals. However, little information is available on soluble conjugate phenolics and their antioxidant activity in rice bran.

A number of studies have demonstrated phenolic compounds typically occur in the insoluble bound form, rarely in the free form, in edible plants (Ahmad, Zuo, Lu, Anwar, & Hameed, 2016). For example, 74% of the total phenolics in rice are in the insoluble bound form. Furthermore, the antioxidative properties of bound phenolics are significantly higher than in free or soluble conjugated forms (Adom & Liu, 2002). A subsequent publication from Shao, Xu, Sun, Bao, and Beta (2014) reported that ferulic, *p*-coumaric, syringic, and isoferulic acids are mainly bound in rice bran. Therefore, there is a large amount of interest in finding an effective method to release the bound phenolic compounds. While there are some methods that have found to be successful at releasing phenolics from rice bran, such as subcritical water extraction (Wiboonsirikul et al., 2007), high hydrostatic pressure and far-infrafred radiation (Kim et al., 2015; Wanyo, Meeso, & Siriamornpun, 2014), the disadvantages of these methods, such as high energy consumption, expensive equipment, production on a small scale and low efficiency, limit their use in industry. An alternative approach of obtaining phenolics from rice bran is enzymatic release, which is a low cost method that requires only mild reaction conditions and is environmentally friendly. A recent study found that using a single cellulase treatment on rice bran increased the amount of free phenolic acids, such as protocatechuic and vanillic acids. However, this was a low efficiency process as it only slight increased the amount of free phenolic acids and failed to increase the total amount of phenolics (Wanyo et al., 2014).

The cell wall of rice bran is a complex three dimensional structure consisting of cellulose, polysaccharide and protein (Benoit et al., 2006). One possible method of degrading the cell wall to further release phenolics is complex enzyme hydrolysis using protease, cellulase and glucoamylase. The work presented here focuses on this method of enzymatic release of phenolics from rice bran.

The objectives of this study were as follows: (1) to determine the contents of free and soluble conjugate phenolics and flavonoiods and their antioxidant activities of rice bran extract at different stages of treatment; (2) to characterize changes in the compositions and contents of individual phenolics in the both free and soluble conjugate forms in rice bran extract during the enzymatic process.

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), fluorescein disodium salt, and 2,20-azobis(2-amidinopropane)dihydrochloride (ABAP) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC grade acetonitrile and acetic acid were obtained from Fisher (Suwanee, GA, USA). Individual phenolic standards were purchased from Aladdin Reagents (Shanghai, China). Alpha-amylase (20,000 uints/g), glucoamylase (100,000 uints/g), acid protease (50,000 uints/g) and acid cellulase (35,000 uints/g) were food grade and purchased from Youtell Biochemical Com., Ltd (Shanghai, China). All solvents used in chromatography were of HPLC grade and other chemicals were of analytical reagent grade.

2.2. Complex enzyme hydrolysis of rice bran

The fresh rice bran used in this study had been defatted using supercritical carbon dioxide (Rice Research Institute of Guangdong Academy of Agricultural Sciences, China) and was made up of 52.87% carbohydrate, 29.66% starch, 16.72% protein, 23.21% crude fiber and 12.35% ash based on dry weight (DW).

Complex enzyme hydrolysis was performed as described in a previous report with modifications (Wen et al., 2015). Briefly, 10 g rice bran were added into 50 mL of 0.01 M citric buffer (pH 6.0), and gelatinization was performed by heating the solution to 100 °C for 10 min. This mixture was cooled and kept at 70 °C in a thermostatic water bath. Liquefaction was then performed by adding alpha-amylase to the mixture and incubating for 10 min. The mixture was heated to 90 °C for 5 min to stop the liquefaction reaction, and the pH was adjusted to 4.1 using citric acid. Complex enzyme hydrolysis was then performed in triplicate, where this mixture was incubated with a complex of enzymes consisting of 0.5% glucoamylase, 1.5% protease and 1.5% cellulase, based on the weight of rice bran, for 190 min at 57.5 °C. Samples from each of the three stages were collected and phenolics were extracted and quantified.

2.3. Extraction of free phenolics

Free phenolic compounds were extracted according to a previously reported method, with modifications (Alrahmany, Avis, & Tsopmo, 2013). Briefly, 5 g rice bran extract was dissolved in 50 mL of acidified water (pH 3.0) and partitioned five times with 50 mL of ethyl acetate. The pooled ethyl acetate fractions were evaporated to dryness. The extract containing the free phenolics was reconstituted with MeOH to a final volume of 10 mL and then stored at −20 °C until analysis.

2.4. Extraction of soluble conjugate phenolics

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

2.5. Determination of total phenolic content

The total phenolic content in both the free and soluble conjugate fractions was measured by the Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, a 125 μL aliquot of the above extract described above was diluted with 0.5 mL distilled water, and subsequently mixed with 125 μL Folin–Ciocalteu reagent. After 6 min, 1.25 mL 7% aqueous sodium carbonate solution was added, and the solution was diluted to a final volume of 3 mL. The reaction solution was incubated in dark for 90 min, and the absorbance was measured 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 (GAE) per 100 g dry weight (DW) of rice bran.

2.6. Determination of total flavonoid content

The total flavonoid content was determined according to the colorimetric method with minor modifications (Min, Gu, McClung, Bergman, & Chen, 2012). A 300 μL aliquot of the above extract was mixed with 1.5 mL distilled water, and subsequently with 90 μL 5% $NaNO_2$ solution. After 6 min, 180 μL 10% $AlCl_3{\cdot}6H_2O$