Application of Germination and Enzymatic Treatment to Improve the Concentration of Bioactive Compounds and Antioxidant Activity of Rice Bran

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Abstract: Rice bran has been recognized as an excellent source of bioactive compounds, but only a small amount is consumed by humans. The limitation of using rice bran in a food industry is its rough texture and low concentration of bioactive compounds, when incorporated into food products. The purpose of this study was to improve the concentration of bioactive contents and antioxidant activity of rice bran, through the use of germination and enzymatic treatment. Rough rice was germinated prior to drying and milling to obtain germinated rice bran (GRB), whereas the regular rice bran (RB) was obtained by milling the ungerminated rough rice. In order to prepare a rice bran enzymatic treatment, GRB and RB were dissolved in distilled water prior to adding with neutral protease and alpha amylase. The slurry was sieved, heated and freeze-dried to obtain a rice bran extracted powder. The results indicated that both germination of rough rice and enzymatic treatment improved the levels of ß-oryzanol (452.21 mg 100g⁻¹), α-tocopherol (26.14 mg 100g⁻¹) and phenolic contents (743.74 mg GAE 100g⁻¹) by 2, 3 and 5 times, respectively, compared with that of untreated rice bran, whilst only enzymatic treatment, but germination, increased the concentration of protein, reducing sugar and total amino acid of the rice bran. Four different methods were used to evaluate the antioxidant activity of the rice bran. The germination and enzymatic extraction improved the antioxidant activity of rice bran products, which those obtained from enzymatic treatment (RBE and GRBE) revealed the strongest antioxidant activity.

Key words: rice bran, germinated, enzyme, bioactive compounds, antioxidant activity

INTRODUCTION

Rice bran is a by-product from the milling process of rice, which contains approximately 10-12 % of total kernel weight, including pericarp, seed coat, nucellar layer, aleurone layer, embryo and the outer portion of the starchy endosperm (Rohrer and Siebenmorgen, 2004; Lee et al., 2005; Da Silva et al., 2006). Rice bran has been recognized as an excellent source of nutrients and bioactive compounds, such as ß-oryzanol, tocopherols and tocotrienols, which offer beneficial health properties and antioxidant activity (Ryynanen et al., 2004). A number of studies have reported on the health benefits of tocopherols, tocotrienols and ß-oryzanol, such as lowering the risk of cancer formation, coronary heart diseases and cholesterol (Rohrer and Siebenmorgen 2004; Imsanguan et al., 2007), having anti-inflammatory activity and inhibiting cholesterol oxidation (Rong et al., 1997; Akihisa et al., 2000; Xu et al., 2001). Antioxidants can inhibit or retard the oxidation of an oxidisable substrate in a chain reaction. The potent antioxidant helps to protect biological molecules and tissue from oxidative damage (Yoshida et al., 2003). In addition to these health benefits, the antioxidant in rice bran has a potential use as an additive, which can improve the storage stability of a variety of food (Nanua et al., 2000; Kim and Godber, 2001; Feblez et al., 2002; Garcia et al., 2007; Watchararuchi et al., 2008; Sereeawthanawut et al., 2008).

Although rice bran is an excellent source of biochemical compounds, only a small proportion is consumed by humans or processed to produce rice bran oil. Its application, as a source of bioactive compounds or antioxidant, is an alternative that adds value to the rice bran. However, its coarse texture and a low...
concentration of the bioactive compounds limit its application and incorporation into food products. Various methods have been developed to enhance the level of bioactive components in food materials, including thermal, alkali, acid and chemical treatments. In this study, germination and enzymatic treatments have been proposed, since an enzyme causes only minimal changes to original food compositions and therefore it minimizes the loss of important components (Parado et al., 2006), whereas germination can improve the concentration of nutrients and phytochemicals (Palmiano and Juliano, 1972). During the germination of a plant seed, enzymes are activated, which hydrolyze biological components, such as starch, non-starch polysaccharides and proteins, which then give rise to an increase in oligosaccharides, simple sugars, peptides, and amino acids in plant seeds, for example, in barley (Rimsten et al., 2003) and wheat (Yang et al., 2001). The decomposition of the high molecular weight polymers, during germination, leads to the generation of bio-functional substances and the improvement of organoleptic qualities, due to the softening of texture and an increase in the barley’s flavour (Beal and Mottram, 1993), oats (Heinio et al., 2001), finger millet (Rao and Muralikrishna, 2002) and brown rice. During the germination process of brown rice, nutrients in the rice change significantly, such as γ-aminobutyric acid (GABA), dietary fiber, inositol, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitor (Kayahara et al., 2000). Several bioactive compounds are antioxidant, such as oryzanol, tocopherols, tocotrienols, and phenolic compounds, which are mostly located in the bran layer. Therefore, a germination process and a partial enzymatic treatment were applied as the alternative methods, in order to enhance the concentration of chemical components, major bioactive compounds (total phenolic compound, γ-oryzanol and α-tocopherol) and the antioxidant activity of the rice bran.

**MATERIALS AND METHODS**

**Materials:**

Protease type II from *Aspergillus oryzae* (0.140 units/mg), α- amylase (E.C. 3.2.1.1) type XII-A from *Bacillus licheniformis* (500 KU), standard α- tocopherol and Low-density lipoprotein (LDL) from human plasma were purchased from Sigma-Aldrich Chemical Co., (St. Louis, Mo, USA). HPLC grade methanol, acetonitrile, hexane, ethyl acetate and ethanol were purchased from BHD (Poole, UK). γ- Oryzanol standard was purchased from Tsuno food industrial Co., Ltd. (Wakayama, Japan) Gallic acid, 1, 1-diphenyl-2- picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were obtained from Fluka Chemical (Buchs, Switzerland). All chemicals and reagents were an analytical grade.

**Methods:**

**Preparation of Rice Bran (RB) and Germinated Rice Bran (GRB):**

**Rice Bran Preparation:**

Rough rice of *Oryza sativa* L., cultivar RD-6 (crop year 2007) was purchased from a local rice-milling factory in Mahasarakham province, Thailand. The rough rice was dehusked and polished by a dehusker and a rice milling machine, respectively, in order to obtain the rice bran.

**Germinated Rice Bran Preparation:**

The germination method followed the method reported by Saetang (2007). The rough rice was soaked in water at room temperature for 48 h and the water was changed every 6 h. The soaked sample was then placed in a plastic box, with cheesecloth placed at the bottom and the top. The germination took place in a germination cabinet, at 28±2°C, and the relative humidity was controlled at 90±2% for 48 h. The germinated rice was dried in a tray drier (45±2°C), to reach a final moisture content of 10-12%, prior to dehusking and polishing to obtain the germinated rice bran. RB and GRB were cleaned, ground, sieved, passed through a 20 mesh sieve and heated at 100 °C for 15 minutes, using an autoclave to inactivate lipase (Juliano, 1985). The samples were stored at -20 °C, until used.

**Preparation of Partial Rice Bran Treatments Using Enzymes:**

The method of partial treatment by enzyme followed the method of Odibo et al., (2002) and Parado et al., (2006), with some modifications to the preliminary study. RB and GRB of 200g were dissolved in 1,000 mL of distilled water. The temperature and pH of the slurry were adjusted to 37°C and pH 7.5, before a neutral protease was added and stirred for 30 minutes. Then the temperature and pH of the slurry were adjusted to 65°C and pH 6.9, before adding α- amylase and shaking it in a shaker water bath for 60 minutes. The slurry was homogenized (Ultra-Turrax T50 homogenizer, USA), passed through 40 mesh sieve, heated at 85±2 °C
for 10 minutes and then freeze dried, in order to obtain the rice bran extract powder (RBE) and germinated rice bran extract powder (GRBE). The samples were stored at -20°C until analysis.

**Preparation of Rice Bran and Germinated Rice Bran Treatment Using Water (RBW and GRBW):**
The rice bran and germinated rice bran were also extracted with water using similar steps to that used with the enzymes except that, in this case, enzymes were not used.

**Determination of Proximate Compositions of Rice Bran Products:**
The rice bran products were analyzed for crude protein, crude fat, crude fiber, ash, nitrogen free extracts, free amino acid (AOAC, 2000) and reduced sugar (Somogi, 1952)

**Sample Extraction for the Analysis of Bioactive Compounds and Antioxidant Activity:**
The extraction was carried out according to the method reported by Iqbal et al., (2005). The samples (5.0g, dry weight) were extracted with 25 mL of 80% methanol for 3 h in an electrical shaker, at room temperature. The samples were further extracted twice with 20 mL of 80% methanol containing 0.15% HCL, under the same set of conditions. The extracts were filtered through Whatman No.1 filter paper and evaporated to dryness under reduced pressure at 45°C, by a rotary evaporator (Buchi, Switzerland). This extract was dissolved with 5 mL of methanol and used for the analyses of total phenolic compound and antioxidant activity.

**Determination of Total Phenolic Compound:**
The total phenolic compound (TPC) of the rice bran products was determined by Folin-Ciocalteu reagent (Iqbal et al., 2005). The reaction was initiated by mixing 0.2 mL of appropriate diluted rice bran extracts, 0.8 mL of freshly prepared diluted Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The final mixture was diluted to 7 mL with deionized water. The mixture was kept in the dark at an ambient temperature for 2 h. The absorbance at 765 nm was measured, using a UV-Vis spectrophotometer (Shimadzu, Japan). The results of the total phenolic compounds were expressed as mg gallic acid equivalents (GAE) per g of rice bran.

**Determination of γ-Oryzanol and α-Tocopherol:**
The content of the rice bran products were measured (with some modifications) according to the method reported by Ryynanen et al., (2004) and Chen and Bergman (2005). The rice bran (0.5g dry weight) was accurately weighed into a glass tube. Ascorbic acid (0.1g), ethanol (5 mL) and water (2 mL) were added. After mixing the tube with a vortex mixer, KOH (0.5 mL) was added. The tube was capped and transferred to a boiling water bath for 25 minutes and mixed by the vortex mixer every 5 minutes, while boiling. The tube was cooled in an ice-water bath and then 2.5 mL of water and 2.5 mL of ethanol were added to the cooled tube. An unsaponified layer was extracted three times, using 10 mL of the mixture of n-hexane and ethyl acetate (8:2). The tube was shaken for 10 minutes and the organic layers were collected, using a separation funnel. These organic extracts were washed three times with water and evaporated by a rotary evaporator (Buchi, Switzerland). The residue was dissolved in methanol. Prior to HPLC analysis, the extracts were filtered through a 0.45mm syringe filter. An analysis of γ- oryzanol and α-tocopherol was performed, using the reversed phase high performance liquid chromatography (RP-HPLC), according to the method reported by Chen and Bergman (2005), with some modifications. The Shimadzu HPLC system (model L-6200A), equipped with a Photo diode array detector (Shimadzu, Japan) and a computer system, was applied. Detection was operated at 292 and 325 nm, simultaneously. The spectra, from 250 to 600 nm, were recorded for all peaks. The extracted samples were injected through a guard-column and separated on a C18 column (4.60 x 150mm, 4 μm) (Phenomenex, USA). Gradient elution was then applied. Mobile phases A, B, and C were methanol, water and butanol, respectively. The gradient was as follows: 0-12 min 92% A, 4% B and 4% C: 12-25 min linear gradient, from 4% B to 3 % B and 4% C to 5 % C, with flow rate of 1.5 mL /min and injection volume of 20 mL. The α-tocopherol was detected at 292 nm and γ-oryzanol was detected at 325 nm. Chromatograms were recorded, and peak areas were used to calculate the content of γ-oryzanol and α-tocopherol, against the standard curve of standards.

**Evaluation of Antioxidant Activity:**
**DPPH Radical Scavenging Activity:**
The antioxidant activity of the rice bran products was determined, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The method described by Dasgupta and De (2004) was applied. The extracts (0.1 mL) were added to 3 mL of 0.004 % methanolic solution of DPPH. The control
sample contained 0.1 mL methanol without extract. BHT and α-tocopherol were used as the standard antioxidant for comparison. The absorbance at 517 nm was measured, using a UV-visible spectrophotometer (Shimadzu, Japan) after incubation for 30 minutes in the dark, at room temperature. The percent inhibition activity was calculated as: 

\[
\frac{(A_0 - A_e)}{A_0} \times 100
\]

\(A_0 = \text{Absorbance without extract} ; \ A_e = \text{Absorbance with extracts}\)

The radical scavenging activity of the rice bran extract was expressed as the concentration of the RB required for 50% inhibition of free radical (IC_{50} g mL^{-1}).

**Linoleic Acid Emulsion System-thiocyanate Method:**

The Linoleic acid emulsion system-thiocyanate method, described by Suja et al., (2005), was used to evaluate the antioxidant activity of the rice bran products. The reaction mixture was made up of 0.28g linoleic acid, 0.28g of Tween 20 and 50 mL of phosphate buffer (0.2M, pH 7.0), 2.5 mL of the linoleic acid emulsion, 0.5 mL of test sample and 2.5 mL of phosphate buffer (0.2 M, pH 7.0) were mixed and incubated at 37 °C for 120 h. This mixture was prepared by using 0.5 mL of methanol to replace the test sample, which was the control. Readings were taken every 24 h, by taking 0.1 mL of the mixture and then mixing it with 5.0 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 35% HCl. This mixture was reacted at room temperature for 3 minutes and the absorbance was measured at 500 nm.

**Total Antioxidant Activity:**

The determination of total antioxidant activity was performed according to the method reported by Dasgupta and De (2004). The extracts (0.3 mL) were combined with 3 mL of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The mixtures were incubated at 95 °C for 90 minutes. After the mixture was cooled to room temperature, the absorbance of the solution was measured at 695 nm, against a blank. The antioxidant activity is expressed as the number of equivalents of BHA.

**Inhibition of LDL Oxidation:**

The inhibition of the LDL oxidation of the extract was determined according to the method reported by Yu et al., (2005). A solution of human LDL containing of EDTA was dialyzed in a 100-fold volume of 0.01 mol L^{-1} phosphate buffer solution (PBS) (pH 7.4) containing 0.16 mol L^{-1} NaCl to remove EDTA. The buffer was changed every 6 h during 24 h. The stock protein solution of 200 μg per mL was prepared with PBS, stored under nitrogen at 4 °C in the dark and used within 24 h for the LDL oxidation assay. The LDL oxidation was initiated by mixing the LDL solution, containing SDS at a concentration of 10g L^{-1}, with 20 μL of the DMSO solution of the rice bran extract (using 20 μL of DMSO as control) and then a freshly prepared copper chloride solution was added. The final CuCl₂ concentration was 15μmol L^{-1} and the final LDL content was 100 μg of protein per mL, in all assay mixtures. The total volume of each assay mixture was 1.0 mL. The oxidation reaction was carried out at 20 °C for 60 minutes. The level of lipid oxidation in the assay mixture was then measured, by measuring the thiobarbituric acid reactive substances (TBARS). TBARS were determined by the addition of 1 mL of TBAR reagent, in each of the assay mixtures. The resulting solution was heated in a boiling water bath for 30 minutes. After cooling to 20 °C, absorbance at 532 nm was determined and used to calculate the TBARS, using a standard curve prepared with 1,1,3,3-tetraethoxypropane.

**RESULTS AND DISCUSSION**

**Effect of Germination and Enzymatic Treatment on Proximate Compositions of Rice Bran Products:**

The rice bran products obtained were a light brown powder which the appearance was similar to that of rice bran flour. The chemical compositions of the rice bran products are presented in Table 1. Investigation into the effect of germination, on the chemical compositions of the rice bran, revealed that germination had a significant effect on the crude protein, carbohydrate and reducing sugar content. The influence of enzymatic treatments on the chemical compositions of the rice bran products indicated that the use of enzymes yielded a higher level of protein, reducing sugar and total free amino acid content, than the untreated rice bran. The highest values of total amino acids were 17.86 % in GRBE and 13.50 % in RBE and the lowest was RB (10.62 %). The improvement of crude protein and free amino acids, during germination and enzymatic extraction, could be due to some enzymes and nucleic acid is produced and the increase in protease activity accounts for the increase in the level of soluble protein and amino acids (Palmiano and Juliano, 1972). Moreover, Protease added during the extraction of the rice bran digested, solubilized and hydrolyzed the initial
insoluble proteins, which gave rise to an increase of soluble proteins, peptides and free amino acids in the extract (Parrado et al., 2006). The decrease of carbohydrate in GRBE and RBE was also due to the hydrolysis of some starch content by α- amylase added to the rice bran and yield reducing sugar. Moreover, during germination, the starch content of the endosperm was decreased dramatically as α- amylase increased in activity, in the rice grain. Therefore, the enzymatic extraction contained more reduced carbohydrate, than those in the other extractions (Qureshi et al., 2002). The germination and enzymatic extraction did not affect the concentration of crude fat and crude fiber (p< 0.05). The values of crude fat and crude fiber obtained in this study ranged between 10.70 -13.91 % and 10.18 -14.21%, respectively. The results were similar to those reported by Chotimarkorn et al., (2008).

Effect of Germination and Enzymatic Treatment on Bioactive Compounds:

The TPC of untreated rice bran was 223.6 mg 100g⁻¹. This result was similar to that reported by Chotimarkorn et al., (2008) in which the TPC values ranged from 220 to 320 mg gallic acid equivalent 100 g⁻¹rice bran, in different cultivars of raw rice bran. The germination of rough rice affected the total phenolic content of the rice bran, as indicated in Table 2. This result was similar to that reported by Tian et al., (2004), who studied germinated brown rice. Changes of TPC content, in the germinated plant seeds, was dependent on several factors, such as the variety and type of phenolic compounds and the germination conditions (Fernandez-Orozco et al., 2008). After treatment using enzymes, it was found that enzyme treatment was able to efficiently improve the concentration of TPC, compared with those of the untreated rice bran. The highest TPC was shown in RBE with an amount of 836.21 mg 100 g⁻¹, followed by that in GRBE. This may be due to the effect of enzymatic hydrolysis, which liberates and frees phenolic components and increases the level of TPC. Tian et al., (2004) reported that, in rice grain, phenolic compounds were mainly in insoluble form. However, after germination, a decrease of major soluble phenolic compounds was observed, such as hydroxycinnmate sucrose esters, whilst some types of phenolic compound increased, such as ferulic acid. Moreover, the germination of the rough rice significantly increased the concentration of γ- oryzanol and α-tocopherol of the rice bran (p<0.05). The highest level of γ- oryzanol was found in the GRBE (452.21 mg 100g⁻¹), followed by GRBW (301.63 mg 100 g⁻¹) and the lowest was found in RB (207.16 mg 100 g⁻¹). Similar results were observed in the concentration of α- tocopherol. The germination caused a progressive increase in γ- oryzanol and α- tocopherol by 29.31% and 125%, respectively, compared with those that were ungerminated. After enzymatic treatment, the concentration of γ- oryzanol and α- tocopherol was raised by 118.29% and 318.91%, respectively, compared with those that were ungerminated. The level of tocopherols was affected by germination, as reported by Fernandez-Orozco et al., (2009) in chick pea. However, germination could reduce some homolog of tocopherol, such as γ- and δ-tocopherol.

Table 1: Explanation of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>Untreated RB</td>
<td>Untreated rice bran</td>
</tr>
<tr>
<td>GRB</td>
<td>Rice bran obtained from germinated rough rice (germinated rice bran)</td>
</tr>
<tr>
<td>RBW</td>
<td>Rice bran treated using water</td>
</tr>
<tr>
<td>GRBW</td>
<td>Germinated rice bran treated using water</td>
</tr>
<tr>
<td>RBE</td>
<td>Rice bran treated using enzymes</td>
</tr>
<tr>
<td>GRBE</td>
<td>Germinated rice bran treated using enzymes</td>
</tr>
</tbody>
</table>

Table 2: Chemical composition of rice bran extracts (% dry weight)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Untreated RB</th>
<th>GRB</th>
<th>RBW</th>
<th>GRBW</th>
<th>RBE</th>
<th>GRBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.58 ± 0.70a</td>
<td>12.28 ± 0.44b</td>
<td>7.33 ± 0.52a</td>
<td>8.93 ± 0.69b</td>
<td>9.55 ± 0.56c</td>
<td>11.71 ± 0.44a</td>
</tr>
<tr>
<td>Crude protein</td>
<td>10.17±0.79b</td>
<td>12.78±0.65b</td>
<td>10.94 ± 0.71a</td>
<td>13.95±0.54b</td>
<td>14.63± 0.44a</td>
<td>13.85± 0.51b</td>
</tr>
<tr>
<td>Crude fat</td>
<td>13.04± 0.06a</td>
<td>13.01± 1.38b</td>
<td>13.47± 1.05a</td>
<td>13.91± 1.11a</td>
<td>12.48± 1.82a</td>
<td>13.70± 0.96b</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>45.99± 2.14a</td>
<td>40.96±1.32b</td>
<td>40.32± 1.46a</td>
<td>40.21±1.48b</td>
<td>36.63± 1.89b</td>
<td>36.80± 1.40b</td>
</tr>
<tr>
<td>Ash</td>
<td>11.72±0.35a</td>
<td>10.69±0.97b</td>
<td>12.87± 0.76a</td>
<td>12.23±0.80b</td>
<td>12.48±0.49b</td>
<td>13.66± 0.59b</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>1.38± 0.22a</td>
<td>3.64± 0.15b</td>
<td>2.98± 0.17a</td>
<td>6.10± 0.35a</td>
<td>8.95± 0.17a</td>
<td>10.52± 1.54a</td>
</tr>
<tr>
<td>Free amino acid</td>
<td>10.62±1.11a</td>
<td>11.29±0.81b</td>
<td>11.28± 0.72a</td>
<td>11.51±1.98b</td>
<td>13.50±0.73a</td>
<td>12.86± 0.42a</td>
</tr>
</tbody>
</table>

Each Mean ± SD represents three replications.

Means within a row with different superscript letters (a, b, c, d) are different (p<0.05).

Effect of Germination and Enzymatic Treatment on Antioxidant Activity:
DPPH Radical Scavenging:

The scavenging activity of rice bran products determined by DPPH radical was dependent on antioxidant in the rice bran, which interacted with DPPH radical by either giving an electron or hydrogen atom to DPPH radical, thus neutralizing its free radical character (Dasgupta and De (2004). The DPPH radical scavenging was expressed as IC₅₀ value. The antioxidant activity of the rice bran treated by enzymes was stronger, than those treated with water extraction or no treatment (Fig. 1). The strongest antioxidant activity (IC₅₀) of 34.86 mg mL⁻¹ was observed in GRBE, 36.44 mg mL⁻¹ in RBE and 39.95 mg mL⁻¹ in GRBW. This was due to germination and the treatments, which increased the antioxidant compounds, such as phenolic compounds, γ-oryzanol, and α-tocopherol and thus improved the antioxidant activity of GRBW and RBE.

Linoleic Acid Emulsion System-thiocyanate Method:

The antioxidant activity of the rice bran products, to prevent peroxidation of linoleic acid, was evaluated by the thiocyanate method in the emulsion system. The results are shown in Fig 2. The highest percentage of inhibition, of all samples, was indicated at the incubation time of 72 h. The enzymatic treated rice bran samples were expressed as having a more protective effect than others. This may be due to the higher content of bioactive compounds, which corresponded to the antioxidant activity. The reaction of the radical scavenging of the control was decreased, due to the oxidation of linoleic acid hydroperoxides, which can decompose to several secondary oxidation products. These oxidized products reacted with ferrous sulphate to form ferric sulphate, which further reacted with ammonium thiocyanate, to form the ferric thiocyanate and this yielded a red color. Antioxidant in the rice bran samples could have retarded the oxidation of the linoleic acid and therefore, the formation of ferric thiocyanate would have been slow (Suja et al., 2005).

Total Antioxidant Activity:

The total antioxidant activity of the rice bran products were expressed as the number which was equivalent to the antioxidant standards (BHA, vitamin C and gallic acid) (Table 3). The assay was based on the reaction of Mo (VI) to Mo (V) to the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (Dasgupta and De, 2004). In this study, the total antioxidant capacity of the rice bran treated by using enzymes significantly increased (p<0.05) the number of equivalents of all the antioxidant standards. The enzymatic treated rice bran demonstrated electron-donating capacity and thus they may have acted as radial chain terminators, which transformed the reactive free radical species into more stable non-reactive products (Arabshahi-Delouee and Urooj, 2007).

Fig. 1: Antioxidant activity of rice bran determined by DPPH radical scavenging assay. The values are Means ± standard deviation (SD), n = 3 expressed as the concentration of rice bran required to scavenge DPPH radical by 50% (IC₅₀, unit = mgmL⁻¹).
Fig. 2: Antioxidant activity of rice bran determined in the linoleic acid emulsion system-thiocyanate method. Values are Means ± SD, n = 3 expressed as % inhibition compared with the antioxidant activity of emulsion (time = 0 h) prior to adding the extracts and BHA.

Fig. 3: Antioxidant activity determined by LDL oxidation. Values are Means ± SD, n = 3 expressed as μg TBARS per g of sample per 100 μg of protein extracted from the LDL.

Table 3: Bioactive compounds of the rice bran products (mg 100g\(^{-1}\) dry weight)

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC-content (mg 100g(^{-1}))</th>
<th>γ-oryzanol</th>
<th>Content (mg 100g(^{-1}))</th>
<th>Increase (%)</th>
<th>α-tocopherol</th>
<th>Content (mg 100g(^{-1}))</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated RB</td>
<td>223.16±66 (^*)</td>
<td>0</td>
<td>207.16</td>
<td>0</td>
<td>6.24 ± 0.22</td>
<td>0</td>
<td>6.24 ± 0.22</td>
</tr>
<tr>
<td>GRB</td>
<td>216.32±13 (^*)</td>
<td>-3.14</td>
<td>267.88 (±0.11)</td>
<td>29.31</td>
<td>14.06</td>
<td>± 0.16</td>
<td>125.32</td>
</tr>
<tr>
<td>RBW</td>
<td>281.74±12 (^*)</td>
<td>26.01</td>
<td>231.76 (±0.35)</td>
<td>11.87</td>
<td>15.05</td>
<td>± 0.16</td>
<td>141.03</td>
</tr>
<tr>
<td>GRBW</td>
<td>290.88±15 (^*)</td>
<td>30.04</td>
<td>301.63 (±6.68)</td>
<td>45.60</td>
<td>27.57</td>
<td>± 0.24</td>
<td>373.88</td>
</tr>
<tr>
<td>RBE</td>
<td>836.21±19 (^*)</td>
<td>173.21</td>
<td>252.99 (±5.23)</td>
<td>22.61</td>
<td>27.77</td>
<td>± 0.71</td>
<td>505.13</td>
</tr>
<tr>
<td>GRBE</td>
<td>743.74±34 (^*)</td>
<td>566.37</td>
<td>452.21 (±8.80)</td>
<td>118.29</td>
<td>26.14</td>
<td>± 0.05</td>
<td>318.91</td>
</tr>
</tbody>
</table>

Each Mean ± SD represents three replications
Means within columns with different superscript letters (a, b, c, d) are different (p<0.05).
* % increase was compared with the content of untreated rice bran.
Table 4: Antioxidant activity evaluated by total antioxidant method (μg antioxidant standard equivalent mg⁻¹)

<table>
<thead>
<tr>
<th>Sample</th>
<th>BHA</th>
<th>Vitamin C</th>
<th>Gallic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated RB</td>
<td>2.17 ± 0.36 e</td>
<td>3.10 ± 0.56 e</td>
<td>0.40 ± 0.48 e</td>
</tr>
<tr>
<td>GRB</td>
<td>8.06 ± 0.15 e</td>
<td>12.82 ± 0.25 e</td>
<td>1.03 ± 0.01 e</td>
</tr>
<tr>
<td>RBW</td>
<td>3.31 ± 0.39 e</td>
<td>4.91 ± 0.62 e</td>
<td>0.54 ± 0.46 e</td>
</tr>
<tr>
<td>GRBW</td>
<td>28.34 ± 1.85 e</td>
<td>50.06 ± 3.54 e</td>
<td>2.54 ± 0.12 e</td>
</tr>
<tr>
<td>RBW</td>
<td>125.66 ± 0.72 e</td>
<td>250.15 ± 1.56 e</td>
<td>7.51 ± 0.03 e</td>
</tr>
<tr>
<td>GRBW</td>
<td>154.44 ± 2.82 e</td>
<td>313.31 ± 6.19 e</td>
<td>8.65 ± 0.12 e</td>
</tr>
</tbody>
</table>

Each Mean ± SD represents three replications. Means within columns with different superscript letters (a, b, c, d, e) are different (p<0.05). The concentration of sample was 0.2g mL⁻¹.

Inhibition of LDL oxidation:

LDL oxidation involves both lipid and protein fraction, through different mechanisms. A free radical-mediated chain reaction is a possible mechanism involved in LDL oxidation, resulting in secondary products (thiobarbituric acid reactive substance, TBARS). The lower TBARS indicates the reduction of lipid peroxidation in the LDL solution (Yu et al., 2005). In this study, the rice bran products significantly reduced (p<0.05) the TBARS production in LDL. The greatest reduction was indicated by the lowest level of TBARS production. The greatest reduction was observed in GRBE (8.52 μg TBARS g⁻¹ sample 100 μg⁻¹ protein) and RBE (8.97 μg TBARS g⁻¹ sample100 μg⁻¹ protein). The chelating potency of antioxidants reduced the availability of transition metals, including Cu²⁺ as catalysts to generate the radicals that initiate the oxidative chain reaction and directly react with and convert the peroxides to less reactive components (Yu et al., 2005).

Conclusions:

This study suggests that germination of rough rice and the enzymatic treatment of rice bran are effective methods to improve the concentration of bioactive compounds and antioxidant activity. Therefore, these rice bran products may be exploited as a potent source of bioactive compounds and antioxidants, for nutraceutical and functional food products.

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REFERENCES


