

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

¹J. Sungsopha, ¹A. Moongngarm, ²R. Kanesakoo

¹Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44000 Thailand.

²Department of Biotechnology Technology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000 Thailand.

Abstract: Rice bran has been recognized as an excellence 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 γ -oryzanol ($452.21 \text{ mg } 100\text{g}^{-1}$), α -tocopherol ($26.14 \text{ mg } 100\text{g}^{-1}$) and phenolic contents ($743.74 \text{ mg GAE } 100\text{g}^{-1}$) 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 γ -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 γ -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; Febles *et al.*, 2002; Garcia *et al.*, 2007; Watchararujj *et al.*, 2008; Sereewatthanawut *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

Corresponding Author: Anuchita Moongngarm, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand.
Tel: +66-43-743-135, Fax: +66-43-743-135
E-mail: anuchitac@yahoo.co.th

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, inositols, 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 $28\pm 2^\circ\text{C}$, and the relative humidity was controlled at $90\pm 2\%$ for 48 h. The germinated rice was dried in a tray drier ($45\pm 2^\circ\text{C}$), to reach a final moisture content of 10-12%, prior to dehussing 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 $85\pm 2^\circ\text{C}$

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 Ryyanen *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 μ m) (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: $[(A_0 - A_e) / A_0] \times 100$

(A_0 = Absorbance without extract; A_e = 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 $CuCl_2$ concentration was 15 $\mu mol L^{-1}$ 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 hydroxycinnamate 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 had no treatment. The concentration of γ -oryzanol of untreated RB was higher than the amount documented by Igbal *et al.*, (2005) and Chotimarkorn *et al.*, (2008) (12 to 38 g 100 g⁻¹). This could be due to many factors, such as rice cultivar, the growing environment and postharvest treatment. 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

Abbreviation	Definition
Untreated RB	Untreated rice bran
GRB	Rice bran obtained from germinated rough rice (germinated rice bran)
RBW	Rice bran treated using water
GRBW	Germinated rice bran treated using water
RBE	Rice bran treated using enzymes
GRBE	Germinated rice bran treated using enzymes

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

Composition	Untreated RB	GRB	RBW	GRBW	RBE	GRBE
Moisture	8.58 ± 0.70 ^c	12.28± 0.44 ^a	7.33 ± 0.52 ^d	8.93± 0.69 ^{bc}	9.55 ± 0.56 ^b	11.71± 0.44 ^a
Crude protein	10.17±0.79 ^c	12.78±0.65 ^b	10.94 ± 0.71 ^c	13.95±0.54 ^{ab}	14.63± 0.44 ^a	13.85± 0.51 ^{ab}
Crude fat ^{ns}	13.04 ± 0.06	13.01 ± 1.38	13.47 ± 1.05	13.91 ± 1.11	12.48 ± 1.82	13.70 ± 0.96
Carbohydrate	45.99± 2.14 ^a	40.96±1.32 ^b	40.32 ± 1.46 ^b	40.21±1.48 ^b	36.63± 1.89 ^c	36.80± 1.40 ^c
Crude fiber ^{ns}	10.18 ± 0.99	10.62 ± 0.86	12.86 ± 0.67	12.77 ± 1.48	11.21 ± 1.01	12.29 ± 0.84
Ash	11.72±0.35 ^b	10.69±0.97 ^b	12.07 ± 0.70 ^b	12.23±0.80 ^b	12.48±0.49 ^b	13.66± 0.59 ^a
Reducing sugar	1.38 ± 0.22 ^c	3.64 ± 0.15 ^d	2.99 ± 0.17 ^d	6.10 ± 0.35 ^c	8.95 ± 0.17 ^b	10.52± 1.54 ^a
Free amino acid	10.62±1.11 ^b	11.29±0.81 ^b	11.28 ± 0.72 ^c	11.51±1.98 ^{ab}	13.50± 0.73 ^a	12.86± 0.42 ^a

Each Mean ± SD represents three replications.

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

^{ns} = Means within a row are not different

Carbohydrate (nitrogen free extract) = 100 - (%moisture + % crude protein + % crude fat + % ash + % crude fiber)

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 radical chain terminators, which transformed the reactive free radical species into more stable non-reactive products (Arabshahi-Delouee and Urooj, 2007).

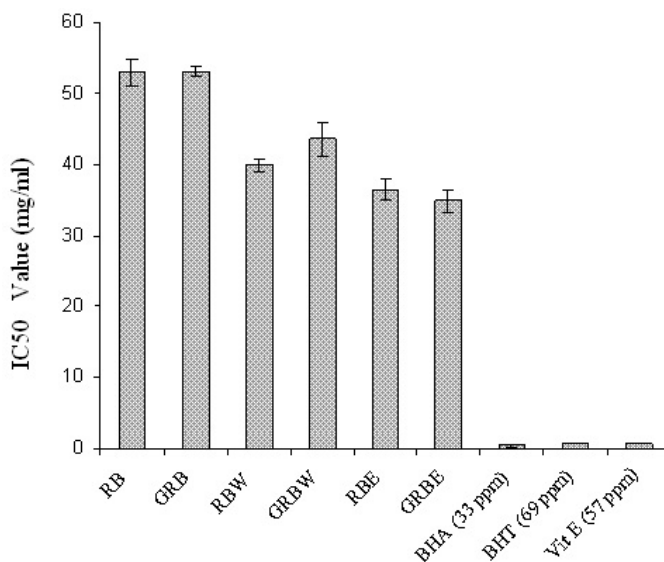


Fig. 1: Antioxidant activity of rice bran determined by DPPH radical scavenging assay. The values are Means \pm standard deviation (SD), n = 3 expressed as the concentration of rice bran required to scavenge DPPH radical by 50% (IC₅₀, unit = mgmL⁻¹).

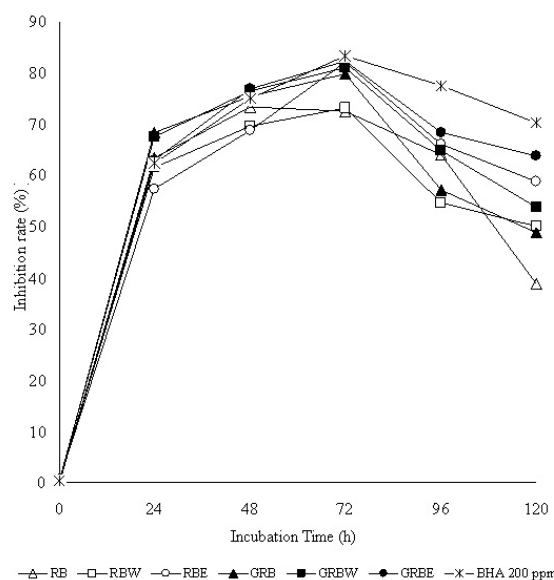


Fig. 2: Antioxidant activity of rice bran determined in the linoleic acid emulsion system-thiocyanate method. Values are Means \pm SD, n = 3 expressed as % inhibition compared with the antioxidant activity of emulsion (time = 0 h) prior to adding the extracts and BHA.

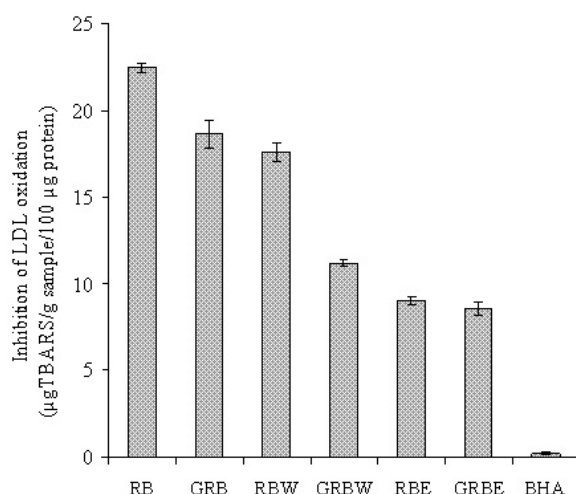


Fig. 3: Antioxidant activity determined by LDL oxidation. Values are Means \pm SD, n = 3 expressed as μg TBARS per g of sample per 100 μg of protein extracted from the LDL.

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

Sample	TPC		γ -oryzanol		α -tocopherol	
	Content($\text{mg } 100\text{g}^{-1}$)	Increase (%)*	Content ($\text{mg } 100\text{g}^{-1}$)	Increase (%)	Content ($\text{mg } 100\text{g}^{-1}$)	Increase (%)
Untreated RB	223.16 \pm 66 ^c	0	207.16	0	6.24 \pm 0.22 ^d	0
GRB	216.32 \pm 13 ^d	-3.14	267.88 \pm 0.11 ^c	29.31	14.06 \pm 0.16 ^c	125.32
RBW	281.74 \pm 12 ^c	26.01	231.76 \pm 0.35 ^d	11.87	15.05 \pm 0.35 ^b	141.03
GRBW	290.88 \pm 15 ^c	30.04	301.63 \pm 6.68 ^b	45.60	27.57 \pm 0.24 ^a	373.88
RBE	836.21 \pm 19 ^a	476.21	253.99 \pm 5.23 ^c	22.61	27.77 \pm 0.71 ^a	505.13
GRBE	743.74 \pm 34 ^b	566.37	452.21 \pm 8.80 ^a	118.29	26.14 \pm 0.05 ^a	318.91

Each Mean \pm 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})

Sample	Total antioxidant capacity		
	BHA	Vitamin C	Gallic acid
Untreated RB	2.17 ± 0.36^c	3.10 ± 0.56^c	0.40 ± 0.48^c
GRB	8.06 ± 0.15^d	12.82 ± 0.25^d	1.03 ± 0.01^d
RBW	3.31 ± 0.39^c	4.91 ± 0.62^{dc}	0.54 ± 0.46^c
GRBW	28.34 ± 1.85^c	50.06 ± 3.54^c	2.54 ± 0.12^c
RBE	125.66 ± 0.72^b	250.15 ± 1.56^b	7.51 ± 0.03^b
GRBE	154.44 ± 2.82^a	313.31 ± 6.19^a	8.65 ± 0.12^a

Each Mean \pm 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 0.2 g 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 ($8.52 \mu\text{g TBARS g}^{-1}$ sample $100 \mu\text{g}^{-1}$ protein) and RBE ($8.97 \mu\text{g TBARS g}^{-1}$ sample $100 \mu\text{g}^{-1}$ protein). The chelating potency of antioxidants reduced the availability of transition metals, including Cu^{2+} , 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.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Mahasarakham University, Thailand and The National Research Council of Thailand (NRCT) for their financial support.

REFERENCES

- Akihisa, T., H. Yasukawa, M. Yamaura, M. Ukiya, Y. Kimura, N. Shimizu and K. Arai, 2000. Triterpene alcohol and sterol ferulates from rice bran and their anti-inflammatory effects. *Journal of Agricultural and Food Chemistry*, 48(6): 2313-2319.
- AOAC, 2002. Official Methods of Analysis. Association of Official Analytical Chemist International .17th Ed., AOAC International, Gaithersburg, MD, USA,
- Arabshahi-Delouee, S. and A. Yrooj, 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chemistry*, 102(4): 1233-1240.
- Beal, A.D. and D.S. Mottram, 1993. An evaluation of the aroma characteristics of malted barley by free choice profiling. *Journal of the Science of Food and Agriculture*, 61(1): 17-22.
- Chen, M.H. and C.J. Bergman, 2005. A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ -oryzanol contents. *Journal of Food Composition and Analysis*, 18(4): 319-331.
- Chotimarkorn, C., S. Benjakul and N. Silalai, 2008. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. *Food Chemistry*, 111(3): 636-641.
- Dasgupta, N. and B. De, 2004. Antioxidant activity of *Piper betle* L. leaf Extract in vitro. *Food Chemistry*, 88(2): 219-224.
- Da Silva, M. A., C. Sanches and E. R. Amante, 2006. Prevention of hydrolytic rancidity in rice bran. *Journal of Food Engineering*, 75(4): 487-491.
- Febles, C. I., A. Arias, A. Hardisson, C. Rodríguez-Álvarez and A. Sierra, 2001. Phytic acid level in infant flours. *Food Chemistry*, 74(4): 437-441.
- Febles, C. I., A. Arias, A. Hardisson, C. Rodríguez-Álvarez and A. Sierra, 2002. Phytic acid level in wheat flour. *Journal of Cereal Science*, 36(1): 19-23.

- Fernandez-Orozco, R., J. Frias, H. Zielinski, M.K. Piskula, H. Kozłowska, C. Vidal-Valverde, 2008. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emmerald, *Glycine max* cv. jutro and *Glycine max* cv. merit. *Food Chemistry*, 111(3): 622-630.
- García-Estepa, R. M., E. Guerra-Hernández and B. García-Villanova, 1999. Phytic acid content in milled cereal products and breads. *Food Research International*, 32(3): 217-221.
- Heinio, R.L., Oksman-Caldentey, K.M., Latva-Kala, K., Lehtinen, P., Poutanen, K., 2001. Effect of drying treatment conditions on sensory profile of germinated oat. *Cereal Chemistry*, 78(6): 707-714.
- Imfanguan, P., B.A. Roaysubtawee, R. Borirak, S. Pongamphai, S. Douglas and P. L. Douglas, 2008. Extraction of α -tocopherol and γ -oryzanol from rice bran. *LWT-Food Science and technology*, 41(8): 1417-1424.
- Iqbal, S., M.I. Bhangar and F. Anwar, 2007. Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. *LWT-Food Science and technology*, 40(2): 361-367.
- Juliano, B., 1985. Rice bran. In B. Juliano (Ed.), *Rice Chemistry and technology*. St. Palu, MN: The American Association of Cereal Chemist., p. 659.
- Kayahara, H., K. Tsukahara and T. Tatai, 2000. Flavor, health and nutritional quality of pre-germinated brown rice. In: Spanier, A.M., F. Shahidi, T.H. Parment, C. Mussinan, C.T. Ho, E.T. Contis (Eds.), 10th International Flavor Conference, 4-7 July 2000. Paros, Greece., pp: 546-551.
- Kim, J.S. and J.S. Godber, 2001. Oxidative stability and vitamin E levels increased in restructured beef roasts with added rice bran oil. *Journal of Food Quality*, 24(1):17-26.
- Lee, T.T.T., M.C. Chung, Y.W. Kao, C.S. Wang, L.J. Chen and J.T.C. Tzen, 2005. Specific expression of a sesame storage protein in transgenic rice bran. *Journal of Cereal Science*, 41(1): 23-29.
- Nanua, J. N., J. U. McGregor and J. S. Godber, 2000. Influence of high-oryzanol rice bran oil on the oxidative stability of whole milk powder. *Journal of Dairy Science*, 83(11): 2426-2431.
- Odibo, F.J.C., L.N. Nwankwo and R.C. Agu, 2002. Production of malt extract and beer from Nigerian sorghum varieties. *Process Biochemistry*, 37(8): 851-855.
- Parrado, J., E. Miramontes, M. Jover, J.F. Gutierrez, L. Collantes de Terán and J. Bautista, 2006. Preparation of a rice bran enzymatic extract with potential use as functional food. *Food Chemistry*, 98(4): 742-748.
- Palmiano, E.P. and B.O. Juliano, 1972. Biochemical Changes in the Rice Grain during Germination. *Plant Physiology*, 49(5): 751-756.
- Qureshi, A., S. Sami and F. Khan, 2002. Effects of Sterilized Rice Bran, Its Soluble and Fiber Fractions on Blood Glucose Levels and Serum Lipid Parameters in Humans with Diabetes Mellitus Types I and II. *J. Nutritional Biochemistry*, 13(3): 175-187.
- Rimsten, L., T. Stenberg, R. Andersson, A. Andersson, and P.Aman, 2003. Determination of beta-glucan molecular weight using SEC with calcofluor detection in cereal extracts. *Cereal Chemistry*, 80(4):485-490.
- Rao, M., G. Muralikrishna, 2002. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (*ragi*, *Eleusine coracana* Indaf-15). *Journal of Agricultural and Food Chemistry*, 50(4): 889-892.
- Rohrer, C.A. and T.J. Siebenmorgen, 2004. Nutraceutical concentrations within the bran of various rice kernel thickness fractions. *Biosystems Engineering*, 88(1): 453-460.
- Rong, N., L.M. Ausman and R.J. Nicolosi, 1997. Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters. *Lipids*, 32(3): 303-309.
- Ryynänen, M., A.M. Lampi, P. Salo-Väänänen, V. Ollilainen and V. Piironen. 2004. A small-scale sample preparation method with HPLC analysis for determination of tocopherols and tocotrienols in cereals. *Journal of Food Composition and Analysis*, 17(6): 749-765.
- Saetang, N., 2007. Study on Nutrients and Bioactive Compounds in Germinated Rice, M.S. Thesis. Mahasarakham University, Mahasarakham, Thailand.
- Sereewatthanawut, I., S. Prapintip, K. Watchirarujji, M. Goto, M. Sasaki and A. Shotipruk, 2008. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. *Bioresource Technology*, 99(3): 555-561.
- Somogyi, M., 1952. Notes on sugar determination. *Journal of Biological Chemistry*, 195(1): 19-23.
- Suja, K.P., A. Jayalekshmy and C. Arumughan, 2005. Antioxidant activity of sesame cake extract. *Food Chemistry*, 91(2): 213-219.
- Tian, S., K. Nakamura, H. Kayahara, 2004. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *Journal of Agricultural and Food Chemistry*, 52(15): 4808-4813.

Watchararujj, K., M. Goto, M. Sasaki and A. Shotipruk, 2008. Value-added subcritical water hydrolysate from rice bran and soybean meal. *Bioresource Technology.*, 99(14): 6207-6213.

Xu, Z.M., N. Hua and J.S. Godber, 2001. Antioxidant activity of tocopherols, tocotrienols, and gamma-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis(2-methylpropionamide) dihydrochloride. *Journal of Agricultural and Food Chemistry.*, 49(4): 2077-2081.

Yang, F., Basu, T.K. and B. Ooraikul, 2001. Studies on germination conditions and antioxidant contents of wheat grain. *International Journal of Food Sciences and Nutrition.*, 52(4): 319-330.

Yoshida, Y., E. Niki and N. Noguchi, 2003. Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. *Chemistry and Physics of Lipids.*, 123(1): 63-75.

Yu, L., K. Zhou and J. W. Parry, 2005. Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. *LWT - Food Science and Technology.*, 38(5): 463-470.