

ANTIOXIDANT ACTIVITY OF JASMINE RICE BRAN HYDROLYSATE AND ITS APPLICATION IN BROKEN RICE BEVERAGES

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Received: May 01, 2020; Revised: June 18, 2021; Accepted: June 29, 2021

Abstract

The objectives of this work were to determine the optimum pH and hydrolysis time for the production of Jasmine rice bran hydrolysate (RBH) with high antioxidant activity using 2% Alcalase. In addition, the antioxidant activity of RBH produced on a laboratory scale and scaled-up production were compared. The results showed that the optimum pH and hydrolysis time were 10.0 and 120 min, respectively. At 120 min, ABTS radical scavenging activity of RBH produced in scale-up was not different when compared with laboratory scale. The RBH prepared at 120 min was used to study the optimum ratio of maltodextrin in producing RBH powder with a high yield of α -amino content, total phenolic content, and antioxidant activity. After spray drying 2 L of RBH mixed with various concentrations of maltodextrin (0-20% w/v), the RBH mixed with maltodextrin 20% (RBH_20) exhibited the highest yield of α -amino content (4,766 mg Leucine eq.), total phenolic content (136 mg Gallic acid eq.), ABTS radical scavenging activity (5,098 mg Trolox eq.) and ferric reducing antioxidant power (1,704 mg Trolox eq.). Therefore, RBH_20 was used to determine the molecular weight (MW) distribution and antioxidant activity in broken rice beverage systems. The major peak of MW peptides from RBH_20 was 837-7462 Da, followed by 621 Da. ABTS radical scavenging activity of broken rice beverages supplemented with RBH_20 increased with increasing concentrations of RBH_20. These results suggested that rice bran hydrolysate mixed with 20% maltodextrin could be used as an ingredient in functional beverages with antioxidant properties.

Keywords: Rice bran hydrolysate, Antioxidant activity, Maltodextrin, Spray drying, Broken rice beverages

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Introduction

The Tongsumrit area contains 14 districts and 113 sub-districts, and is an important area for growing rice in Nakhon Ratchasima, Thailand. Jasmine rice is the main strain which is grown in this area at about 70%, namely Tongsumrit Jasmine rice. Phimai Agriculture Cooperative Limited is an important center that collects and buys un-milled rice from farms. The rice milling industry results in an abundance of co-products, namely broken rice and rice bran. Rice bran is used as feed for animals with low value, while broken rice was sold to private companies at a very cheap price. A means to fully utilize and increase marketability of rice bran and broken rice should be sought.

Rice bran contains high quality protein, namely albumin, globulin, prolamin and glutelin around 12-20% (Wattanasiritham *et al.*, 2016). Numerous reports suggested that peptides derived from enzymatic hydrolysis of rice bran display antioxidant activity. Antioxidant activity of rice bran hydrolysate (RBH) depends on hydrolysis conditions (enzyme used, pH, temperature, and hydrolysis time). The enzyme type had significant effects on the protein yield and the properties of rice bran hydrolysate (RBH). Alcalase was the most effective at providing RBH with the highest protein yield and protein content (Thamnaratip *et al.*, 2016). Peptides in RBH with smaller MW possessed higher antioxidant activities. Tyrosine and phenylalanine had positive correlations with their ABTS radicals scavenging activities and ferric reducing antioxidant power (Phongthai *et al.*, 2018). Most of the researchers produced RBH with antioxidant on a laboratory scale. However, antioxidant activity of RBH might be changed in scaled-up production. Therefore, the antioxidant activity of RBH produced on a laboratory scale in comparison with scaled-up production should be determined. Beside scaling-up the production process, drying technologies might affect antioxidant activity of RBH.

Among the drying methods, lyophilization or freeze drying is well-known for preserving quality and the integrity of bioactive compounds in food matrices; however, it has operational costs higher than spray-drying (Schulze *et al.*, 2014; Haque and Adhikari, 2015; Ozorio *et al.*, 2019). Spray-drying is a rapid, continuous, cost-effective, reproducible and scalable process for the production of dry powders (Sosnik and Seremeta, 2015). Maltodextrin, a common encapsulating material used in the food industry. It can act as bulking agent in food powder in spray drying process, and can be added to beverages to improve the mouth feel.

During spray drying, sticky products can be produced, thereby adhering to the internal wall of drying chamber, leading to a lower yield (Hennigs *et al.*, 2001). Addition of maltodextrin can protect against this stickiness problem. Therefore, the optimum ratio of maltodextrin to RBH on the yield of peptide content and antioxidant activity of RBH powder should be considered.

Broken rice is one of the largest co-products in the rice industry as mentioned above. Rice flour constitutes 8.93% protein, 1.41% fat, 4.47% moisture, and 0.52% Ash (Ahmed *et al.*, 2016). Broken rice mainly contained high levels of carbohydrates and low level of protein. To increase the value of broken rice, it should be transformed into a functional beverage by the addition of RBH having substantial antioxidant activity.

Therefore, the objectives of this work were to determine the optimum pH and hydrolysis time for production of protein hydrolysate from Tongsumrit Jasmine rice bran with antioxidant activity using 2% Alcalase on a laboratory scale compared to scaled-up production, and to determine the effect of maltodextrin addition on yield of peptide content and antioxidant activity of RBH powder. Furthermore, antioxidant activity of RBH supplemented in broken rice beverages was also investigated.

Materials and Methods

Materials

Tongsumrit Jasmine rice bran and broken rice were donated by Phimai Agriculture Cooperative Limited, Nakhon Ratchasima, Thailand. 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2, 4, 6-tripyridyl-s-triazine (TPTZ) Trinitrobenzenesulfonic acid (TNBS) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade.

Preparation of Rice Bran

Rice bran powder was defatted using isopropanol with a ratio of powder to solvent of 1:3, at 40°C, for 20 min, which was repeated a total of two times. Defatted samples were left at room temperature for 2 h, vacuum-packed and kept at -20°C until used.

Proximate Composition

Moisture, crude fat, crude protein, and ash of rice bran and defatted rice bran powder were determined according to AOAC (2000).

Rice Bran Hydrolysate Preparation in Laboratory Scale

Rice bran hydrolysate was prepared by mixing 1 g of defatted rice bran (DRB) powder with 10 ml of DI water in a centrifuge tube and pH adjusted to 9.0 or 10.0 using NaOH. The mixture was hydrolyzed using Alcalase with the ratio of enzyme to substrate of 2: 100 (w/w) at 50°C in water bath for 0, 30, 60, 90 and 120 min. Subsequently, the mixture was heated at 100°C for 10 min to terminate the reaction and centrifuged at 10,000 g for 20 min at 4°C. The supernatants were referred as rice bran hydrolysates (RBHs) and used for determination of α -amino content and ABTS radical scavenging activity.

Determination of α -Amino Content

α -Amino content of RBHs was determined using the trinitrobenzenesulfonic acid (TNBS) method according to Adler-Nissen (1979). L-leucine was used as a standard. Absorbance was monitored at 420 nm using a spectrophotometer (SmartSpec™ Plus, Bio-Rad Laboratories, Hercules, CA, U.S.A.). Results were expressed as mg leucine equivalents/g defatted rice bran (DRB) powder.

ABTS Radical Scavenging Activity Assay

An ABTS radical scavenging activity assay was performed according to Wiriyaphan *et al.* (2012). ABTS⁺ stock solution was prepared by mixing 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution, in 10 mM phosphate buffer (pH 7.4), and kept in the dark for 16 h. Fresh ABTS⁺ working solution was prepared by mixing ABTS⁺ stock solution in 10 mM phosphate buffer (pH 7.4) to attain absorbance of 0.700 ± 0.02 , at 734 nm. Twenty microliters of RBHs were mixed with 1980 μ l of fresh ABTS⁺ working solutions. Then, the reaction mixture was kept in the dark for 5 min and absorbance was monitored at 734 nm. Results were expressed as mg Trolox equivalents/g defatted rice bran (DRB) powder.

Production of Rice Bran Hydrolysate (RBH) in Scale-up

Rice bran hydrolysate on a scaled-up production quantity was prepared under optimum pH. By mixing 4 kg of defatted rice bran powder with 40 L of RO water and adjusted pH to 10.0. The mixture was hydrolyzed using Alcalase with the ratio of enzyme to substrate of 2: 100 (w/w) at 50°C for 120 min in the mixers with stirring blades. Samples were collected at 0, 30, 60, 90 and 120 min and were heated at 100°C for 10 min to terminate the reaction and centrifuged at 10,000 g for 20 min at 4°C. The supernatants were used for

determination of α -amino content and ABTS radical scavenging activity (as described above) and ferric reducing antioxidant power.

Ferric Reducing Antioxidant Power Assay (FRAP Assay)

Reducing power of RBHs was measured using the ferric reducing antioxidant power (FRAP) assay according to Wiriyaphan *et al.* (2012). Briefly, FRAP reagent contained 25 ml of 300 mM acetate buffer pH 3.6, 2.5 ml of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 2.5 ml of 20 mM Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution. One hundred microliters of RBHs at various hydrolysis times were allowed to react with 1000 μ l of fresh FRAP reagent and incubated at 37°C for 15 min in a water bath. Absorbance was measured at 593 nm. Results were expressed as mg Trolox equivalents/g defatted rice bran (DRB) powder. RBH exhibited high ferric reducing antioxidant power and ABTS radical scavenging activity was selected for studying the optimum ratio of maltodextrin in production of RBH powder with high yield of α -amino content, total phenolic compounds and antioxidant activity.

Determination of Optimum Ratio of Maltodextrin for Production of RBH Powder Using Spray Dryer

The optimum ratio of maltodextrin to RBH (0, 5, 10, 15 and 20% (w/v) for production of RBH powder with high yield of α -amino content and antioxidant activity after spray drying was determined. Two liter of RBH at 120 min of hydrolysis time was mixed with different ratios of maltodextrin (0, 100, 200, 300 and 400 g). Then, the mixtures were dried using a spray dryer and used for determination of α -amino content (Adler-Nissen, 1979), total phenolic compounds (Matthaus, 2002), antioxidant activity based on ABTS radical scavenging activity and ferric reducing antioxidant power assays (as described above) and yield of RBHs. RBH powder exhibiting the highest yield of α -amino content, total phenolic compounds and antioxidant activity was used for determination of molecular weight distribution and supplementation in broken rice beverages.

Molecular Weight Distribution

Molecular weight distributions of peptides in RBH and RBH mixed with maltodextrin 20%w/v (RBH_20) were determined using a gel filtration chromatography technique. A one hundred microliters of diluted sample (1.6 mg leucine eq./ml) was loaded in a Superdex Peptide 10/300 GL column (10 mm $\times$ 300 mm; GE Healthcare,

Piscataway, NJ, USA) pre-equilibrated with 30% ACN+0.1% TFA in deionized water. Elution was carried out at a flow rate of 0.6 ml/min using 1 column volumes of 30% ACN+0.1% TFA in deionized water. Absorbance was monitored at 215, 280, and 420 nm. Cytocrom C (12,000 Da), Aprotinin (6,512 Da), AGNQVLNLQADLPK (1480.6 Da), MILLLFR (1,095 Da), PLL (341 Da), LL (244 Da) and tyrosine (181 Da) were used as standard. Blue dextran (2000 kDa) was used to measure the void volume of the column.

Preparation of Broken Rice Flour

Broken rice from Tongsumrit jasmine was roasted at 80-85°C and ground. Ground broken rice was passed through a 125-micron sieve. Then the sample was dried at 180°C in hot air oven for 2 h (moisture content less than 10%). The sample was cooled down to room temperature, packed in plastic bag and kept at refrigerator until used.

Preparation of Broken Rice Beverages

Pasteurized and sterilized broken rice beverages were prepared following Table 1 with addition of 0, 0.2, 0.4, 0.6, 0.8 and 1.0% of RBH mixed with maltodextrin 20%w/v (RBH_20) before pasteurization (65°C, 30 min) and sterilization (121°C, 15 min). Instant powder broken rice beverage was prepared following Table 2. Samples were collected for determination of ABTS radical scavenging activity.

Determination of Antioxidant Activity of RBH in Broken Rice Beverages

A one milliliter of pasteurized and sterilized broken rice beverages was centrifuged at 10,000 rpm for 10 min. Supernatant was collected for ABTS radical scavenging activity assay. Fifteen grams of instant powder broken rice beverages was mixed with 150 ml of hot water (90°C). After 3-5 min, 1 ml of sample was collected and then centrifuged at 10,000 rpm for 10 min. Supernatant was collected for ABTS radical scavenging activity assay.

Statistical Analyses

Data were expressed as means±standard deviations. An independent-sample t-test was used for comparison of the means of two independent group samples. Data were analyzed using analysis of variance (ANOVA) and means comparison was done using Duncan's multiple range test (DMRT) for more than two groups of samples, at 95% confidence level using a PASW Statistics 18 Release 18.0.0 software.

Table 1. Ingredients of pasteurized and sterilized broken rice beverage.

Ingredients	Content (%)
Broken rice flour paste*	50
Sucrose	4
Rice bran oil	1
Calcium carbonate	0.12
Beta-glucan	0.02
Isolate Soy Protein (ISP)	0.5
Fresh Pandanus Palm	2
Tween 80	0.2
Octenyl succinic anhydride (OSA)-modified starches	0.05
Water	42.1

*the ratio of broken rice flour to water was 1: 49

Table 2. Ingredients of instant powder broken rice beverage.

Ingredients	Content (%)
Roasted broken rice flour	62
Creamer	9.4
Sucrose	10.8
Xanthan gum	0.1
Maltodextrin	2
Isolate Soy Protein (ISP)	4.6
Cereal	10.1
Octenyl succinic anhydride (OSA)-modified starches	0.05
Salt	1

Results and Discussion

Proximate Composition

Table 3 shows the proximate composition of rice bran and defatted rice bran from Tongsumrit Jasmine rice. Defatted rice bran contained higher moisture, crude protein and total carbohydrate content, and lower crude fat than that of full fat rice bran ($p<0.05$, Table 3). Ash content of full fat rice bran and defatted rice bran was comparable ($p>0.05$). Crude protein and fat of defatted rice bran were 15.09 ± 0.38 and $2.47\pm0.21\%$, respectively (Table 3). Kumari *et al.* (2018) reported that the crude protein content of the full fat rice bran was $11.00\pm0.37\%$ while defatted rice bran had $13.80\pm0.49\%$ of protein. In addition, full fat rice bran had $18.90\pm0.31\%$ crude fat which was reduced to $0.04\pm0.09\%$ due to removal of fat in defatted rice bran (Kumari *et al.*, 2018). Faria *et al.* (2012) reported that whole rice bran contain total carbohydrate, crud protein, crude lipid, ash and moisture contents of 57.39, 16.61 ± 0.20 , 17.87 ± 0.30 , 8.13 ± 0.04 , and $8.41\pm0.2\%$, respectively. Proximate

composition of RBH in our results were close to those previous reports. These results suggested that rice bran, co-product from the rice milling industry of Phimai Agriculture Cooperative Limited is a rich source of protein that can be utilized by a more efficient means, to produce more value added products.

Table 3. Proximate compositions (% dry basis) of rice bran and defatted rice bran from Tongsumrit Jasmine rice.

Compositions	Content (%)	
	Rice bran	Defatted rice bran
Moisture	12.70±0.25 ^b	18.70±0.51 ^a
Ash ^{ns}	8.93±0.81	10.34±0.79
Crude fat	19.23±0.54 ^a	2.47±0.21 ^b
Crude protein	12.63±0.23 ^b	15.09±0.38 ^a
Total carbohydrate	46.51±0.00 ^b	53.40±0.00 ^a

Note: Total carbohydrate was calculated from formula; 100- (moisture + ash + crude fat + crude protein);

Superscript letters with different letters in the same row indicate a significant difference ($p < 0.05$). ns means not significant difference ($p > 0.05$).

α -Amino Content and Antioxidant Activity of Rice Bran Hydrolysate in Laboratory Scale

α -Amino content of defatted rice bran hydrolysates (RBHs) increased with increasing of hydrolysis time (Figure 1a, $p < 0.05$). This indicates that rice bran protein was hydrolyzed by Alcalase into small peptides and amino acids during hydrolysis. Thamnarathip *et al.* (2016) reported that the hydrolysate yield, protein content and protein yield of rice bran hydrolysates using Alcalase tended to increase with increasing hydrolysis time. α -Amino content of RBHs at pH 10.0 was significantly higher than those obtained from RBHs at pH 9.0 at all hydrolysis times ($p < 0.05$). It could be due to higher solubility and denature of rice bran proteins in pH 10.0 than that of pH 9.0, resulting in more accessible to Alcalase attacks. Wang *et al.* (2015) reported that nitrogen solubility index (NSI%) increased with increasing of pH 6-12. There were researchers who reported that the use of alkaline-solvent (pH 8-12) improved the extraction yield of rice proteins (30-80%) (Phongthai *et al.*, 2018). Maximum protein content in concentrated rice bran protein was 48.53% at pH 10.0, 80 rpm, 300 min of stirring time and 52°C, with an extraction yield of 34.51% (Bernardi *et al.*, 2018). Jiamyangyuen *et al.* (2015) found that optimal extracting conditions were pH 11 and 45 min., resulting in 69.16% protein content and 8.06% yield of rice bran protein concentrate. However, it was observed that a pH higher than 10 demands a huge volume of 2 mol/L NaOH and aqueous solution color changes from light yellow to a greenish tone (Bernardi *et al.*, 2018). In our

experiments, RBH at pH 10.0 and 120 min of hydrolysis time had the highest α -amino content (37.08±1.87 mg Leucine eq./g DRB) (Figure 1a, $p < 0.05$). These results indicated that using alkaline conditions combined with Alcalase could extract peptides from rice bran protein.

ABTS radical scavenging activity was used for screening antioxidant activity of RBHs produced on a laboratory scale because it is simple, inexpensive, and rapid. ABTS radical scavenging activity of RBHs increased with increasing of the hydrolysis time up to 60 min and 90 min, at pH 10.0 and 9.0, respectively and reached plateau after that (Figure 1b, $p < 0.05$). At the end of hydrolysis time, ABTS radical scavenging activity of RBHs was 22.44±0.37 and 25.46±2.22 mg Trolox eq./g DRB at pH 9.0 and 10.0, respectively. ABTS radical scavenging activity of RBHs trend to correlate with α -amino content (Figure 1a). However, at pH 10.0, hydrolysis times at 60-120 min, ABTS radical scavenging activity of RBHs was not significantly different while α -amino content increased with the increasing of hydrolysis time. It indicates that specific amino acids containing in the peptide sequence might be an important factor influencing the ABTS radical scavenging activity. Alcalase had

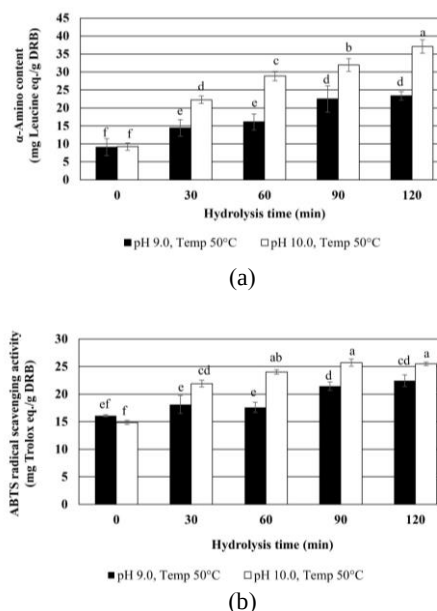


Figure 1. (a) α -Amino content and (b) ABTS radical scavenging activity of rice bran hydrolysates (RBH) treated with Alcalase at 50°C, pH 9.0 and 10.0 at different hydrolysis time produced in laboratory scale. Bars with different letters indicate means with significant differences ($p < 0.05$). Values are expressed as means±S.D. (n=3).

broad specificity, and high specificity toward aromatic (Phe, Trp, and Tyr), acidic (Glu), sulfur-containing (Met), aliphatic (Leu and Ala), hydroxyl (Ser), and basic (Lys) residues (Doucet *et al.*, 2003). Phe, Trp, and Tyr can transfer electrons and/or hydrogen to free radicals (Hernández-Ledesma *et al.*, 2005; Rajapakse *et al.*, 2005; Qian *et al.*, 2008). Therefore, more generated small peptides during hydrolysis times of 60-120 min might not contain antioxidant amino acids in their sequences. RBHs at pH 10.0 contained higher ABTS radical scavenging activity than those obtained from RBHs at pH 9.0 at any hydrolysis times (Figure 1b, $p<0.05$). This could be due to high α -amino content in RBHs at pH 10.0 resulting in higher small peptides than that of pH 9.0 (Figure 1a). These indicated that the optimum pH for production of RBH was pH 10.0. Therefore, pH 10.0 was used as the condition for production rice bran hydrolysate in scaled-up production. However, α -amino content and antioxidant activity produced in the scale-up might be different from laboratory scale production.

α -Amino Content and Antioxidant Activity of Rice Bran Hydrolysate in Scale-up

The α -amino content was released from rice bran protein by Alcalase at a high rate during the initial 30 min and then slowed down thereafter (Figure 2a), which indicated that maximum cleavage of peptides occurred within 30 min of hydrolysis. α -Amino content profile of RBHs produced in the scale-up showed a slight difference from the laboratory scale in which the α -amino content of RBHs in the scale-up was released at a high rate at 30 min, and kept constant after 60 min (Figure 2a) while α -amino content of RBHs in laboratory scale increased with increasing hydrolysis time (Figure 1a). However, at the end of hydrolysis, α -amino content of RBH (30.04 ± 0.43 mg Leucine/g DRB) was slightly lower than that of the laboratory scale (37.08 ± 1.87 mg Leucine eq./g DRB) (Figure 1a).

ABTS radical scavenging activity and ferric reducing antioxidant power of RBHs tend towards increasing with increasing of the hydrolysis time (Figure 2b and Figure 2c). At 90-120 min, ABTS radical scavenging activity and ferric reducing antioxidant power of RBHs increased (Figure 2b and Figure 2c) while α -amino content was constant (Figure 2a). The phenolic compounds contents could be released during enzymatic hydrolysis of rice bran protein (Thammarathip *et al.*, 2016). Therefore, total phenolic compounds in RBHs might be contribute to antioxidant activity. The ABTS radical scavenging activity profile of RBHs produced in pilot scale was slightly different from that produced on a laboratory scale by which

their activity in scaled-up production increased at 30 min, slightly decreased at 60 min, and increased after that (Figure 2b) while activity of RBHs on laboratory scale production increased with increasing hydrolysis time up to 60 min, and kept constant (Figure 1b). However, at the end of hydrolysis, ABTS radical scavenging activity of RBH produced in the scale-up was 27.77 ± 0.14 mg Trolox eq./g DRB (Figure 2b), which was slightly higher than that of the laboratory scale (25.46 ± 2.22 mg Trolox eq./g DRB, Figure 1b). These indicated that ABTS radical scavenging activity of RBH was retained after upscale processing. Thammarathip *et al.* (2016) reported that increasing the hydrolysis time of Alcalase to 4 and 6 h showed slight or non-significant differences in protein yield, protein content, total phenolic compounds

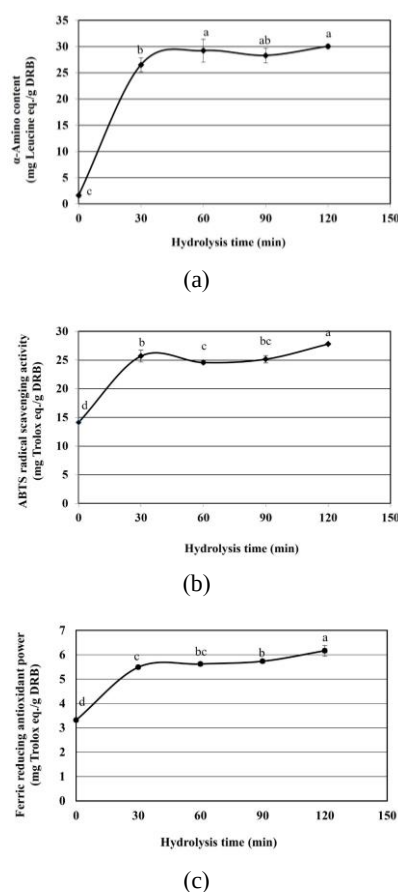


Figure 2. (a) α -Amino content, (b) ABTS radical scavenging activity and (c) Ferric reducing antioxidant power of rice bran hydrolysates (RBH) in upscale treated with Alcalase at 50°C, pH 10.0 at different hydrolysis time produced in scale-up. Bars with different letters indicate means with significant differences ($p<0.05$). Values are expressed as means $\pm$ S.D. (n=3).

and antioxidant activities of rice bran hydrolysates. Authors suggested that the hydrolysis by Alcalase for 2 h could be considered as the optimal condition for commercial food production. Therefore, RBH at 120 min of hydrolysis time was used for the study of the optimum ratio of maltodextrin in production of RBH powder with high yields of α -amino content, total phenolic compounds and antioxidant activity.

Optimum Ratio of Maltodextrin for Production of RBH Powder

After spray drying 2 L of RBHs mixed with various concentrations of maltodextrin (0, 5, 10, 15

and 20% w/v), α -amino content, total phenolic compounds and antioxidant activity of spray dried-powders were determined. The results showed that α -amino content and total phenolic compounds per gram of RBH decreased with increasing the concentration of maltodextrin (Figure 3a and Figure 3b), which correlated well with ABTS radical scavenging activity and ferric reducing antioxidant power (Figure 4a and Figure 4b). Because α -amino content and total phenolic compound in RBHs was diluted with the addition of maltodextrin this resulted in a decreasing of antioxidant activity. Our results were similar to those previous reported by Chuaychan and Benjakul (2016), in which ABTS

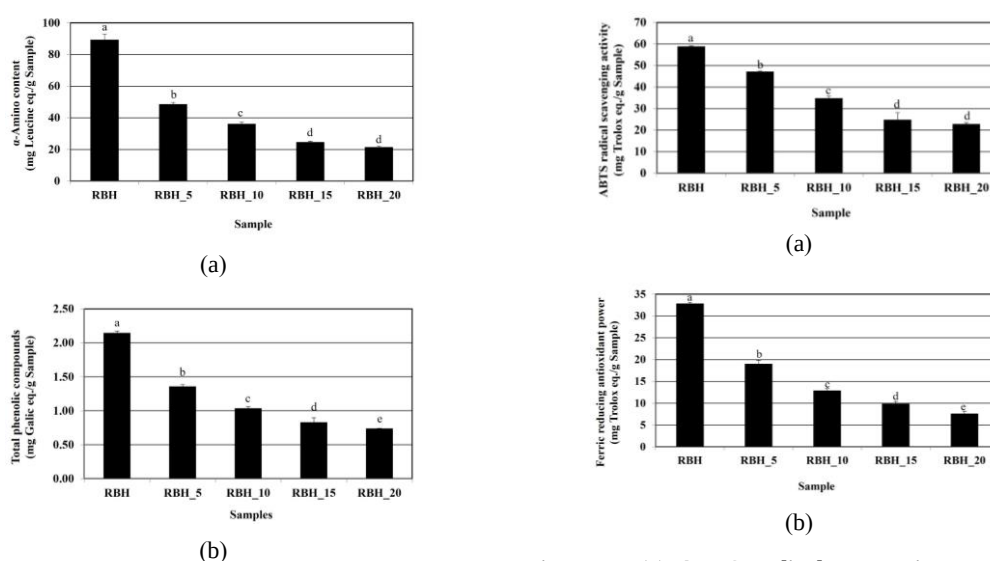


Figure 3. (a) α -Amino acid content and (b) Total phenolic compounds of rice bran hydrolysates (RBH) mixed with various ratio of maltodextrin (0-20% w/v). Bars with different letters indicate means with significant differences ($p < 0.05$). Values are expressed as means $\pm$ S.D. ($n=3$).

Figure 4. (a) ABTS radical scavenging activity and (b) Ferric reducing antioxidant power of rice bran hydrolysates (RBH) mixed with various ratio of maltodextrin (0-20% w/v). Bars with different letters indicate means with significant differences ($p < 0.05$). Values are expressed as means $\pm$ S.D. ($n=3$).

Table 4. Yield of rice bran hydrolysate powder based on total weight, α -amino acid content, ABTS radical scavenging activity and ferric reducing antioxidant power (FRAP) obtained from spray drying of 2 L of rice bran hydrolysate mixed with maltodextrin at various concentrations (0-20% w/v).

Sample	Total weight (g)	Yield			
		Total phenolic compounds (mg Gallic acid eq.) ¹	α -Amino acid content (mg Leucine eq.) ²	ABTS radical scavenging activity (mg Trolox eq.) ³	FRAP (mg Trolox eq.) ⁴
RBH	48	103 $\pm$ 1 ^c	4286 $\pm$ 182 ^b	2830 $\pm$ 10 ^c	1580 $\pm$ 15 ^{ab}
RBH_5	61	82 $\pm$ 2 ^d	2951 $\pm$ 77 ^d	2872 $\pm$ 15 ^c	1158 $\pm$ 49 ^d
RBH_10	113	117 $\pm$ 3 ^b	4072 $\pm$ 171 ^b	3941 $\pm$ 112 ^b	1460 $\pm$ 52 ^{bc}
RBH_15	146	121 $\pm$ 11 ^b	3567 $\pm$ 118 ^c	3617 $\pm$ 476 ^b	1442 $\pm$ 76 ^c
RBH_20	223	164 $\pm$ 1 ^a	4766 $\pm$ 194 ^a	5098 $\pm$ 138 ^a	1704 $\pm$ 116 ^a

¹ Yield was calculated from total weight of RBH powder (g) $\times$ Total phenolic compounds (mg Gallic acid eq./g RBH); ² Yield was calculated from total weight of RBH powder (g) $\times$ α -Amino acid content (mg Leucine eq./g RBH); ³ Yield was calculated from total weight of RBH powder (g) $\times$ ABTS radical scavenging activity (mg Trolox eq./g RBH); ⁴ Yield was calculated from total weight of RBH powder $\times$ Ferric reducing antioxidant power (mg Trolox eq./g RBH)

Superscript letters with different letters in the same column indicate a significant difference ($p < 0.05$).

radical scavenging activities and ferric-reducing antioxidant power of gelatin and gelatin hydrolysate decreased when maltodextrin was used as a carrier agent. However, RBH mixed with 20% maltodextrin (RBH_20) contained the highest yields of α -amino content, total phenolic compounds and antioxidant activity (Table 4). Addition of maltodextrin increased the product yield by preventing the adhesion of sumac (*Rhus coriaria* L.) extract and gelatin hydrolysate inside the chamber walls (Caliskan and Nur Dirim, 2013; Chuaychan and Benjakul, 2016). Addition of 20% maltodextrin in RBH was the optimum ratio for production of RBH powder by using spray drying. Therefore, RBH_20 was used for determination of molecular weight distribution and application in broken rice beverages.

Molecular Weight Distribution

Molecular weight (MW) distribution of RBH and RBH_20 shows the major area peak at 817-7467 Da and 837-7462, with area peaks of 47.45 and 48.02%, respectively (Figure 5a and Figure 5b). Adding 20% of maltodextrin in RBH (RBH_20) slightly increased the area peak of MW distribution at 621-635 Da (from 24.05% to 28.54%), and decreased the area peak of low MW peptides at <224 Da (from 15.99% to 9.71%) (Figure 5b) when compared to RBH without adding of maltodextrin (Figure 5a). This might be due to interaction of maltodextrin and small peptides during the drying process. Carbonyl groups in maltodextrin might undergo Maillard reactions with free amino groups of gelatin hydrolysate at a higher content (Chuaychan and Benjakul, 2016). Therefore, low MW peptides and free amino groups at <224 Da in RBH_20 might interact with carbonyl groups in maltodextrin to form high MW compounds leading to decrease in low MW peptides and amino acid at <224 Da. These results indicated that adding of 20% maltodextrin affected on MW distribution of RBH. Wattanasiritham *et al.* (2016) reported that the MW of the antioxidant peptides from trypsin-hydrolyzed denatured albumin of rice bran protein were 800-2100 Da. and consisted of 6-21 amino acid residues. All three fractions fractionated from rice bran hydrolysate prepared by using Alcalase exhibited high antioxidant activity, which clearly showed a band with an MW of 6 kDa (Thamnarathip *et al.*, 2016). Peptides with high antioxidant activities from rice bran protein hydrolysate were composed of molecular masses ranging from 670 to 3611 Da (Adebiyi *et al.*, 2009) and under 1000 Da (Zhang *et al.*, 2014). The majority of peptides with MW at 1088 Da were supposed to be octapeptides, containing 8 amino acids, which contributed to antioxidant activity of rice bran hydrolysate

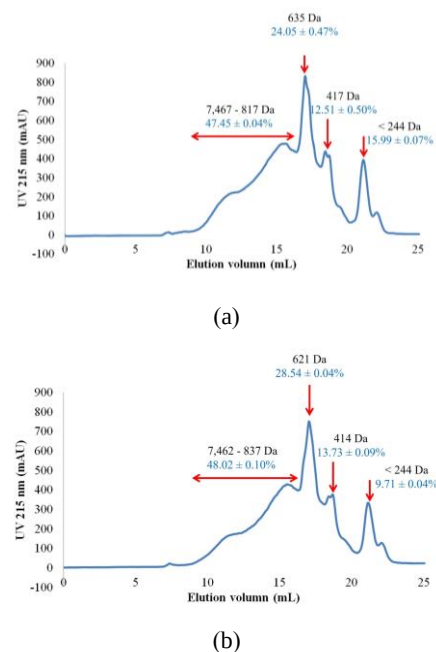


Figure 5. Molecular weight distribution of (a) rice bran hydrolysates (RBH) powder and (b) RBH mixed with maltodextrin at ratio of 20%.

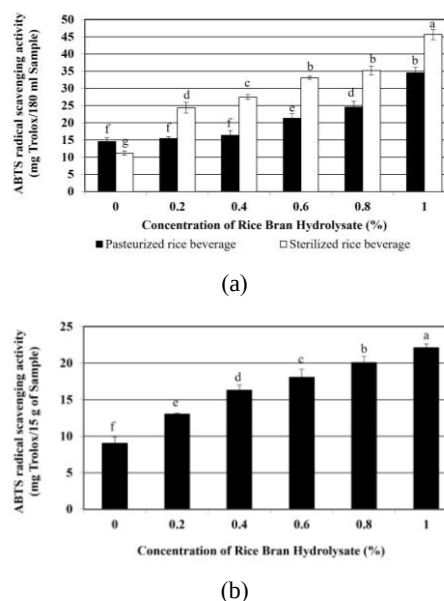


Figure 6. (a) ABTS radical scavenging activity of pasteurized and sterilized and (b) instant powder broken rice beverages supplemented with rice bran hydrolysates mixed with 20% maltodextrin (RBH_20) powder at various concentrations. Bars with different letters indicate means with significant differences ($p < 0.05$). Values are expressed as means $\pm$ S.D. ($n = 3$).

(Phongthai *et al.*, 2018). This study demonstrated that low MW peptides in rice bran hydrolysate might contribute to antioxidant activity.

Antioxidant Activity of RBH_20 in Broken Rice Beverage Systems

The ABTS radical scavenging activity of broken rice beverages increased with increasing the concentration of RBH_20 (Figure 6a and Figure 6b). Low MW peptides and total phenolic compounds in RBH might contribute to antioxidant activity. The ABTS radical scavenging activity of sterilized broken rice beverages was higher than that of pasteurized broken rice beverages at all concentrations. Sterilized, pasteurized and instant powder broken rice beverages supplemented with 1% RBH_20 had the highest ABTS radical scavenging activity of 45.7 and 34.5 mg Trolox eq./180 ml of sample, and 22.1 mg Trolox eq./15 g of sample, respectively. These results indicated that RBH_20 could increase the nutritional value of broken rice beverage with antioxidant activity.

Conclusions

The optimum conditions for the production of rice bran hydrolysate (RBH) with high ABTS radical scavenging activity were a pH of 10.0 and 120 min of hydrolysis time. Production of RBH in scaled-up production had the effect of decreasing the α -amino content, however, ABTS radical scavenging activity was comparable when compared to laboratory scale production. The optimum ratio of maltodextrin was 20% w/v for production RBH powder with a high yield of α -amino content, total phenolic compounds and antioxidant activity by using spray drying. Molecular weight distributions of RBH and RBH mixed with maltodextrin 20% (RBH_20) exhibited the major peak at around 7,467-817 Da, followed by 621-635 Da. Adding of RBH_20 in broken rice beverages could improve their antioxidant activity in a dose-dependent manner. These results suggested that rice bran hydrolysate mixed with 20% maltodextrin prepared using 2% Alcalase might be good source of antioxidant peptides in beverage systems. In future, the suitable condition for RBH production in this research could be applied for large scale RBH production as food additive.

Acknowledgements

This research was financially supported by the Thailand Research Fund (Grant No. RDG59A0014.1-2). Authors would like to thank

Rajamangala University of Technology, Isan for facilities supports.

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