Chemical composition of Kum Bangpra rice bran, rice bran oil and their anthocyanin color powder production

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Abstract The results showed that Kum Bangpra rice bran consisted of proteins (12.85 g/100 g), γ -oryzanol (387.88 mg/100 g), anthocyanin (2.82 mg/100 g), GABA (3.13 mg/100 g), antioxidants (1,253.83 mg ascorbic acid/100 g) and 18 amino acids. The rice bran oil extracted by using isopropanol (yield of 7.40 %) contained higher content of bioactive. Water pH 3.5 was used in anthocyanin extraction and gave an anthocyanin concentration of 6.44 mg/100 g. In addition, the appropriate spray drying condition for the production of anthocyanin color powder was feed rate of 15 ml/min, temperature of 150°C and mixed with maltodextrin (7%). The yield of color powder from the rice bran in this study was 7.7 %. In conclusion, Kum Bangpra rice bran and rice bran oil in this study contained high contents of bioactive compounds and proteins. Their pigment could be extracted and produced into the dry color powder. Kum Bangpra rice bran extract can be a good candidate for further cosmeceutical and nutraceutical product developments. In addition, this is the first report on anthocyanin color powder production from their rice bran.

Keywords: Kum Bangpra rice, Rice bran, Rice bran oil, Anthocyanin, Color powder

Introduction

Rice is a staple food and economic plant of Thailand. The production volume of rice in Thailand was more than 20 tons per year. Pigmented rice is a good natural source of health beneficial bioactive compounds. Rice bran layer contains high amount of nutrients and phytochemicals such as γ -oryzanol, phenolics, tocopherol, tocotrienol, carotenoids, anthocyanins, vitamins, proteins and essential amino acids. These phytochemicals are a source of antioxidants which exhibit bioactivities such as antioxidative, anticancer and anti-inflammatory activities (Pramai and Jiamyangyuen, 2016; Seechamnanturakit *et al.*, 2018; Yamuangmorn and Prom-u-Thai, 2021). Anthocyanins, a group of hydrophilic flavonoids, are the pigments in rice bran layer including red, purple and black colors (Pramai and Jiamyangyuen, 2016; Seechamnanturakit *et al.*, 2018). There are many types of anthocyanins but cyanidin-3-glucoside is the

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major anthocyanin in purple bran (Abdel-Aal *et al.*, 2006). Oil from rice bran also has nutritional and pharmaceutical values. Antioxidants, γ -oryzanol, is the one of the major components in rice bran oil following with tocopherol and tocotrienol as a vitamin E (Dunford, 2019). In addition, rice bran is also a good source of proteins and essential amino acids from cereal grains. Proteins, protein hydrolysates, peptides and amino acids from rice bran are reported to have the health and beauty beneficial activities such as antioxidants (Adebiyi *et al.*, 2008; Thamnarathip *et al.*, 2016), anticancer activity (Kannan *et al.*, 2010; Phusrisom *et al.*, 2021; Wattayagorn *et al.*, 2022), anti-inflammatory activity (Chanput and Lawyer, 2020), antihypertensive activity (Li *et al.*, 2007), Alzheimer's disease preventive activity (El-Din *et al.*, 2021; Hagl *et al.*, 2015), melanogenesis inhibition (Ochiai *et al.*, 2016), antihyaluronidase and antityrosinase activities (Chen *et al.*, 2021).

Kum Bangpra rice (*Oryza sativa* L.) is one of the pigmented rice varieties which is developed from BP2012-009 variety by a bulk selection method (Promsomboon and Promsomboon, 2016; Promsomboon *et al.*, 2018). The un-husked grains and rice bran of Kum Bangpra rice are straw-colored whereas their pericarps are dark purple colored (Figure 1A-1C). This rice contains proteins, starch, fiber, vitamin B, vitamin E, ascorbic acid, γ -amino butyric acid (GABA), γ -oryzanol, anthocyanin, omega 3, amylose, iron, zinc and antioxidative activity (Promsomboon and Promsomboon, 2019). The chemical composition of particular Kum Bangpra rice bran and rice bran oil are not reported. Therefore, the objectives were to evaluate the chemical composition of rice bran and rice bran oil.

Materials and methods

Chemical composition analysis

Kum Bangpra rice bran was analyzed the chemical composition by the Central Laboratory (Thailand) Co., Ltd. for the contents of anthocyanin (AOAC 2005.02 as described in Lee *et al.*, 2005), γ -oryzanol (Chen and Bergman, 2005), proteins (AOAC 981.10 as described in Latimer, 2016), amino acids (method 994.12 as described in AOAC International, 2000), GABA (Posoongnoen and Thummavongsa, 2018), vitamin B2 and B5 (Chen *et al.*, 2006) and antioxidative activity (Lal *et al.*, 2013).

Plant material

Kum Bangpra rice (*Oryza sativa* L.) was collected from Chonburi province, eastern part of Thailand in October 2020. It is developed from

BP2012-009 variety by a bulk selection method (Promsomboon and Promsomboon, 2016; Promsomboon *et al.*, 2018). Rice bran (Kum Bangpra rice) was generated during the first step (Rice bran 1^{st} step) and second step (Full fat rice bran) of milling and obtained from a local milling factory in Sriracha, Chonburi province, Thailand.

Rice bran oil production

Kum Bangpra rice bran was dried by using hot air oven at 110°C for 3 min. Rice bran (500 g) was separately macerated with 1,500 ml of hexane or isopropanol and then placed at room temperature for 7 days. Oil was collected by filtration. The collected oil was eliminated the solvent by using rotary evaporator. The yield of each rice bran oil was calculated.

Chemical composition analysis

Each oil was analyzed their chemical composition by the Central Laboratory (Thailand) Co., Ltd. for the contents of anthocyanin (AOAC 2005.02 as described in Lee *et al.*, 2005), γ -oryzanol (Shammugasamy *et al.*, 2015), γ -tocotrienol (Shammugasamy *et al.*, 2015), total vitamin E (Shammugasamy *et al.*, 2015) and antioxidative activity (Lal *et al.*, 2013).

Quality characteristic analysis

Saponification analysis

Rice bran oil (5 g) was added into a round bottom flask and then 50 ml of 0.5 M KOH in ethanol was added. The flask was refluxed for 30 min to 1 h and then cooled down. The solution was transferred into an Erlenmeyer flask and added with 1 ml of 1% phenolphthalein. Titration was performed by using 0.5 M HCl. A blank was performed in parallel by using water instead of the oil sample. The saponification (S.N.) was calculated as described below. When A is the volume of 0.5 M HCl used in titration of blank, B is the volume of 0.5 M HCl used in titration of blank, B is the volume of 0.5 M HCl used in titration of sample.

S.N. =
$$(A - B) \times 0.5 \times 56$$

W

Iodine analysis

Rice bran oil (1 g) was added with chloroform (10 ml) and iodine monobromide (20 ml). The solution was mixed and then kept at room temperature in the dark for 45 min. A blank was performed in parallel by using

water instead of the sample oil. The solution was added with potassium iodide (10 ml), mixed and then added with distilled water (25 ml). The solution was titrated by using 0.2 M sodium thiosulfate solution until the color of iodine was changed to light yellow-brown. The soluble starch solution (1 ml) was then added resulting in color was changed to blue. The solution was titrated by using 0.2 M sodium thiosulfate solution until the blue color was disappeared. Iodine number was calculated as described below. When N is the concentration of sodium thiosulfate, A is the volume of sodium thiosulfate solution used in titration of oil sample, B is the volume of sodium thiosulfate solution used in titration of blank and W is the weight of oil sample.

Iodine number =
$$\frac{12.69 \times N \times (B - A)}{W}$$

Free fatty acid analysis

Rice bran oil (5 g) was added with diethyl ether (10 ml) and 95% ethanol (10 ml), mixed and then added with 3 to 4 drops of 1% phenolphthalein. The solution was titrated by using 0.5 M KOH. The amount of free fatty acid was calculated as described below. When V is the volume of 0.05 KOH used in titration and W is the weight of oil sample.

Free fatty acid =
$$\frac{0.05 \times 56.1 \times V}{W}$$

Protein analysis

Protein extraction

Defatted rice bran was evaporated for elimination the solvent before extraction of proteins. Rice bran and defatted rice bran were added with deionized water pH 9.55 in the ratio of 1:7, mixed and then placed at room temperature for 30 min. The solution was then separated by using centrifugation at 3,000 rpm/min, 10°C for 10 min. The supernatant was collected, adjusted to pH 4.5 by using 2 M HCl and then placed overnight at refrigerator resulting in the protein precipitation. The precipitated proteins were then collected by using filter paper No. 1.

Protein quantitative analysis

The amount of total protein was analyzed by using Kjeldahl method (method 960.52 as described in AOAC International, 2000). Briefly, Protein sample (1 g) was added into the digestion tube. The tube was then added with CuSO₄ (0.5 g) and K₂SO₄ (10 g) and mixed well. The H₂SO₄ (20 ml) was then gently added and mixed. Blank tube was performed in parallel by using water instead of the sample. The tubes were then placed in protein analyzer. The samples were heated gently for 60 min. The clear solution samples were cooled

down at room temperature, added with 10-20 ml of distilled water and 80 ml of concentrated NaOH and the samples were changed to blackish solution. The tubes were connected to a condenser and then heated until the ammonium was distilled into receiving flask containing 50 ml of boric acid and indicator. The distilled solution was then titrated with standard H_2SO_4 solution. Total protein content was calculated as described below. When V1 is the volume of standard H_2SO_4 used in sample titration, V2 is the volume of standard H_2SO_4 used in blank titration, N is the normality of H_2SO_4 , W is the weight (g) of sample and 1.4 is a constant factor.

Total protein content (%) =
$$\frac{(V1 - V2) \times N \times 1.4}{W}$$

Protein profile analysis

Proteins extracted from Kum Bangpra rice bran was analyzed for their protein profile by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The concentration of extracted proteins was analyzed by using Bradford protein assay and then adjusted to 20 μ g/ml. The 12% separating gel and 4% stacking gel were prepared. The protein sample was loaded and run using 150 V for 2 h. The gel was then stained with Coomassie Brilliant Blue R-250 staining solution for 30 to 60 min. The gel was then destained by using detaining solution for 1 h. The gel was then rinsed by using double distilled water for 30 min. The gel was then dried and analyzed for protein profile by comparing with standard protein marker.

Color powder production

Anthocyanin from rice bran was extracted and analyzed by using modified method from Du and Francis (1973). Briefly, Rice bran (30 g) was added with 60 ml of water pH 3.5, 4.5 or 5.5 which was adjusted by using 1% HCl, mixed and then placed at room temperature for 60 min. The solution was filtered by using filter paper No. 1 and rinsed the rice bran with their solvents. The filtrated solution was adjusted the volume to 100 ml. This solution (5 ml) was added with their solvent (95 ml), mixed and kept in refrigerator for 2 h without light exposure. The solution was then measured the optical density at 528 nm. The anthocyanin content was calculated as described below. When Tcy is total anthocyanin content (mg/100 g), OD528 is optical density value of sample, extinction coefficient of anthocyanin is 55.9.

 $Tcy = OD528 \times dilution \ factor \times sample \ volume \times 100$ sample weight × extinction coefficient

Anthocyanin solution was dried by using spray dryer. The spray drying condition was studied including feed rate of 12 and 15 milliliters per minute, maltodextrin at concentrations of 3%, 5% and 7% and temperature of 150°C. The yield of dried color powder was calculated.

Results

Kum Bangpra rice bran was analyzed for the chemical composition. The results revealed that Kum Bangpra rice bran contained high nutritional and pharmacological values. Result showed the chemical composition of this rice bran which consisted of proteins, γ -oryzanol, anthocyanin, GABA, antioxidants and 18 amino acids (Table 1). Glutamic acid was the highest content of amino acid containing in this rice bran following with aspartic acid, arginine and leucine, respectively. When comparing the results of the study on the constituents of the Kum Bangpra rice bran as shown in Table 1 with those of other purple, purple red and brown rice groups such as Sang Yod rice, Tubtim Chum Phae rice, and Leum Pua glutinous rice. It was found that Kum Bangpra rice bran had higher protein, vitamin B2, antioxidants and γ -oryzanol but lower in anthocyanin and GABA. It was found that the protein of Kum Bangpra rice bran was 12.85 g/100g. The protein content of Sang Yod rice bran, Tubtim Chum Phae rice bran and Leum Pua glutinous rice bran were 8.3, 9.36 and 10.63 g/100g, respectively.



Figure 1. Characteristic of Kum Bangpra rice. A) un-husked grains, B) husked grains and C) rice bran, D) Rice bran oils, E) Anthocyanin extraction and F) Anthocyanin color powder

The rice bran oil was extracted from rice bran by using the extraction solvents, hexane and isopropanol (Figure 1D). The results showed that the rice bran oil extracted by using isopropanol could give higher chemical contents including the antioxidative activity, γ -oryzanol, γ -tocotrienol, total vitamin E (γ -tocotrienol form) and anthocyanin than using hexane (Table 2). Therefore, the rice bran oil extracted by using isopropanol was then selected to analyzed for the quality characteristics and compared with the standard criteria. As shown in Table 3, the values of saponification, iodine number and free fatty acid content were in the range of standard criteria values. These results suggested that the Kum Bangpra rice bran oil extracted by using isopropanol in this study could be met the standard criteria for rice bran quality characteristics.

Table 1. Chemical composition of Kum Bangpra fice bran			
Chemical composition	Content (mg/100 g)		
Anthocyanin (Anthocyanin-3-glucoside)	2.82±0.01		
γ-Oryzanol	387.88±3.02		
Proteins	12,850.00 ±0.18		
Antioxidant (as Ascorbic acid)	1,253.83±0.40		
Vitamin B2	0.09±0.03		
Vitamin B5	0.75±0.13		
γ-Aminobutyric acid (GABA)	3.13±0.11		
Amino acids			
Aspartic acid	1,976.10±5.40		
Threonine	528.92±0.17		
Serine	702.48 ± 1.04		
Glutamic acid	2,171.96±1.71		
Glycine	714.00±0.70		
Alanine	854.69±1.15		
Cysteine	255.28±0.99		
Valine	766.87±0.94		
Methionine	240.36±1.62		
Isoleucine	472.78 ± 1.07		
Leucine	$1,040.48\pm0.77$		
Tyrosine	493.98±1.43		
Phenylalanine	660.08 ± 0.85		
Histidine	430.10±0.70		
Lysine	642.73±1.39		
Arginine	1,113.20±0.14		
Proline	582.85±0.53		
Tryptophan	204.54±0.31		

Table 1. Chemical composition of Kum Bangpra rice bran

Active compounds	Content (mg/100 g)		
_	Hexane	Isopropanol	
γ-Oryzanol	$1,422.53 \pm 1.09^{a}$	1,581.79±0.92 ^b	
γ-Tocotrienol	5.23 ± 0.25^{a}	14.41±0.62 ^b	
Total vitamin E (γ -Tocotrienol form)	6.07 ± 0.20^{a}	15.40±0.53 ^b	
Anthocyanin	0.13 ± 0.03^{a}	0.28 ± 0.07^{a}	
Antioxidative activity (as ascorbic	189.80 ± 1.31^{a}	247.73 ± 1.26^{b}	
acid)			
Yield (%)	6.06 ± 0.37^{a}	7.40±0.52 ^b	

Table 2. Yields of rice bran oils extracted from different solvents and their bioactive compounds

Means in horizontal lines followed by different letter signify statistical difference (P<0.05)

Table 3. Quality characteristics of rice bran oils extracted from isopropanol compared with the standard criteria

Characteristics	Values		
	Isopropanol extraction	Standard criteria*	
Saponification	192±2.64	191 – 195	
Iodine number	95±1.73	92 - 115	
Free fatty acid (%)	3±1.00	<u>≤</u> 4	

*These standard criteria are from Thai Industrial Standards Institute.

The contents of crude proteins extracted from Kum Bangpra rice bran and defatted rice bran were 12.85% and 12.74% and their yields were 8.0% and 7.6%, respectively, suggesting that the proteins still remained in the rice bran even after passing the oil extraction process (Table 4). The concentrated proteins were then analyzed for their protein profile by using SDS-PAGE. The results showed that these concentrated proteins extracted from rice bran had the size in the range of 5 - 40 kDa. The protein bands at 35, 25 and 18 kDa and the small size protein bands at lower than 15 kDa (15 – 5 kDa) were found (Figure 2). These bands might be the peptides that composed in rice bran proteins.

Table 4. Contents of rice bran, defatted rice bran and concentrated rice bran

 proteins and their yields

Samples	Protein content (%)	Yield (%)
Rice bran	12.85±0.88 ^b	8.0 ± 0.20^{b}
Defatted rice bran	12.74±0.94 ^b	7.6 ± 0.40^{b}
Concentrated proteins	37.30±0.84 ^a	1.0 ± 0.10^{a}

Means in the same column, different letters signify statistical difference (P<0.05)

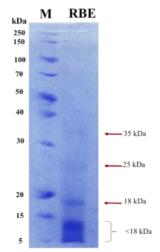


Figure 2. Protein profile of Kum Bangpra rice bran extract by using 12% SDS-PAGE. M represents protein marker, RBE represents rice bran extract

In anthocyanin extraction, the anthocyanin from rice bran was extracted by using water as a solvent. The water was adjusted the pH to 3.5, 4.5 and 5.5. The results showed that the water pH 3.5 gave the higher anthocyanin concentration than that of pH 4.5 and 5.5. The color of this anthocyanin extracted solution was red-orange (Figure 1E). Therefore, the water pH 3.5 was used to extract the anthocyanin and produce the color powder from this rice bran. The spray drying conditions for color powder production were optimized. The results were shown in Table 5, the appropriate spray drying condition was feed rate of 15 ml/min, temperature of 150°C and mixed with maltodextrin (7%). The yield of color powder from the rice bran in this study was 7.7 %. The color of this dried powder was light pink (Figure 1F).

Feed Rate	Extract	Maltodextrin	Color powder	
(ml/min)	(ml)	(% w/v)	Weight (g)	Yield (%)
12	400	3	15.56±0.45 ^a	3.9±0.45 ^a
	400	5	22.57±0.55 ^b	5.6 ± 0.55^{b}
	400	7	$29.15 \pm 1.04^{\circ}$	$7.3 \pm 1.04^{\circ}$
15	400	3	16.15±0.20 ^a	4.0±0.20 ^a
	400	5	24.32±0.43 ^b	6.0±0.43 ^b
	400	7	30.99±0.43°	$7.7 \pm 43^{\circ}$

Table 5. Spray drying conditions and yield of color powder at the temperature of 150°C

Means in the same column, different letters signify statistical difference (P<0.05)

The feed rates of 12 and 15 milliliters per minute did not result in a difference in the amount of pigment. However, using different percentages of maltodextrin resulted in a statistical difference in pigment weight and % yield at P<0.05 without any interaction between factors. (Table 5)

Discussion

Kum Bangpra rice variety contains high nutritional and pharmacological values (Promsomboon and Promsomboon, 2019). These values are correlated with the results of rice bran and rice bran oil in this present study. The chemical composition of Kum Bangpra rice bran in the present study were higher content than that chemical content from the husked grain in the previous study (Promsomboon and Promsomboon, 2019) especially the content of proteins (approximately 1.4 times), γ -oryzanol (approximately 16 times), GABA (approximately 1.5 times) and antioxidants (approximately 12 times). Interestingly, the rice bran oil in this study had higher contents of γ -oryzanol (approximately 770 times), vitamin E (approximately 770 times) and antioxidants (approximately 2.3 times) than that of the husked grain (Promsomboon and Promsomboon, 2019). These results suggested that the rice bran or rice bran oil of Kum Bangpra provided the higher content of these beneficial phytochemicals than the whole husked rice. In addition, the Kum Bangpra rice bran oil could be extracted by using hexane and isopropanol, especially using isopropanol for extraction could give higher nutritional and phytochemical properties than using hexane. The rice bran oil in this study passed a standard criteria of rice bran oil quality based on Thai Industrial Standards Institute's criteria.

The rice bran and rice bran oil from Kum Bangpra in this study possess high antioxidative activity. This activity might due to the antioxidative activity from the bioactive ingredients such as anthocyanin, γ -oryzanol, tocotrienol and vitamin E (Pramai and Jiamyangyuen, 2016; Laokuldilok *et al.*, 2011; Müller *et al.*, 2010). These phytochemicals have health and beauty benefits. Dietary anthocyanins have protective effect against health problems such as cancers, cardiovascular and inflammatory related diseases, liver dysfunction, hypertension and diabetes (Diaconeasa *et al.*, 2020). In addition, anthocyanins had protective activity to skin such as melanoma and UV protection, skin aging and skin damage protection (Rajo *et al.*, 2013; Diaconeasa *et al.*, 2020). Anthocyanins were reported to increase the level of collagen, elastin and hyaluronic acid *in vitro* and *in vivo* (Nanashima *et al.*, 2018). Therefore, anthocyanins are potent bioactive compounds that can be used as an active ingredient in skin products such as anti-aging cream (Abdellatif *et al.*, 2021). In this study, we produced the color powder containing of anthocyanin from Kum Bangpra rice bran. This color powder can be used as an active ingredient together with rice bran extract and/or rice bran oil in cosmetic or cosmeceutical product development.

Rice bran proteins and peptides were reported to have antioxidative and antimelanogenic activities (Ochiai *et al.*, 2016; Zaky *et al.*, 2022). The rice bran hydrolysates and peptides were also reported to exert several biological activities such as antioxidant, antidiabetic, anticancer activities (Liu *et al.*, 2019). Kum Bangpra rice bran proteins in this study, the size in range of 5 - 40 kDa, might also have the same biological activities and could be used as an active ingredient in functional food or cosmeceutical products. However, these proteins will be further studied in their biological activities and formulated into the skincare products.

Kum Bangpra rice bran and rice bran oil in this study contained high contents of bioactive compounds and proteins. Their pigments could be extracted and produced into the dry color powder. Kum Bangpra rice bran extract can be a good candidate for further cosmeceutical and nutraceutical product developments.

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