Utilization of Nongkhai Black Jasmine Rice Bran Oils for Development of Functional Drink Emulsion

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Abstract. Rice bran is a nutritious by-product from rice milling process. It is usually used as animal feed otherwise rejected as waste that could raise environmental issues. This research aims at utilizing oil extracted from Nongkhai black jasmine rice bran to develop an oil-in-water emulsion drink. Oil was extracted from Nongkhai black jasmine rice bran using hexane as a solvent by Soxhlet extraction method. Nongkhai black jasmine rice bran gave an oil yield of 12.38%. The extracted oil contained total phenolic 120.03 ± 1.26 μg GAE/g oil, flavonoid 46.90 ± 0.69 μg QE/g oil and γ-oryzanol 236.73 ± 7.54 μg/g oil. The antioxidant activity of the oil tested by DPPH, ABTS, and FRAP were 1676.63 ± 76.86, 592.75 ± 46.22, 1.31 ± 0.22 μg Trolox equivalent/g oil, respectively. An oil-in-water emulsion beverage was then developed by dispersing 1% (w/w) of Nongkhai black jasmine rice bran oil with 0.1% (w/w) of Tween80/Span80 (1:1 w/w) as emulsifiers in water. The sweetness of the emulsion beverage was adjusted by adding erythritol mixed with stevia extract sweetener at 0.1, 0.2, 0.3, and 0.4 % (w/v). After pasteurization, all emulsion beverages were subjected to Just-about-right and 9-point hedonic sensory tests using a randomized complete block design. The emulsion containing 0.4 % sweetener had the highest overall liking score of 7.0 ± 1.1, representing moderate liking. The emulsion beverage contained γ-oryzanol 13.3 ± 0.3 μg/mL with the antioxidant activity tested by ABTS of 267.0 ± 37.7 μg Trolox equivalent/mL. In conclusion, Nongkhai black jasmine rice bran is a source of bioactive lipids that can be utilized as an ingredient in a plant-based functional drink emulsion.

Keyword. Antioxidant, Functional drink, Nongkhai black jasmine rice bran, Oil-in-water emulsion,

1 Introduction

Rice (*Oryza sativa*) is an important economic crop in Thailand. During the rice milling process, rice bran which is the outer layer of the rice is produced approximately 8-10% of the rice kernel as a by-product. A large amount of rice bran is usually used as a raw material for the production of animal feed otherwise it is discarded as waste that could impact on environmental pollution. The burning of rice bran might release particles related to the PM 2.5 problem that has been a great concern in Thailand [1]. Rice bran possesses high oil content of 18-22%, so it is used as a raw material for rice bran oil processing [2]. Rice bran oil has a ratio of saturated and unsaturated fatty acids similar to the ratio recommended by the World Health Organization for healthy oil consumption [3]. Moreover, rice bran contains lipophilic beneficial substances, including gamma-oryzanol and vitamin E. Pigmented rice bran also contains anthocyanin which is categorized as flavonoid compounds that have antioxidant activity. The types and amounts of these bioactive components vary depending on the varieties of rice. Nongkhai black jasmine rice or RD 83 rice is a variety of rice recently developed in 2019. It has a purple-black seed coat and not sensitive to light. Lasunon *et.al*., (2022) reported that Nongkhai black jasmine rice possessed total phenolic, total flavonoid and anthocyanin contents in the range of 0.010-0.035 g GAE/100 g, 1.5-3.5 g QE/100 g, and, 0.01-0.03 g/100 g, respectively, which decreased during the cooking process [4]. To our knowledge, the bioactive components and antioxidant activities of Nongkhai black jasmine rice bran oil have not been investigated yet.

Functional beverages have grown in related to health concerns among consumers. Along with hydrophilic compounds, various lipophilic bioactive components, such as fat-soluble vitamins, phytosterols, and ω-3 fatty acids, etc. have been extensively reported for their health benefits [5]. Incorporation of these lipid soluble compounds into a beverage of which mainly composed of water is a challenge for food industry. Lipophilic components cannot be distributed steadily in the water phase. Eventually, these non-polar components will aggregate and separate layers on top of the product. Emulsification is an effective technology that mainly applied for emulsion beverage development. Oil-in-water emulsion consists of an oil phase dispersed in a

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water phase in the presence of emulsifier that helps to stabilize the emulsion. In the production of emulsified beverages, more than one type of emulsifier is commonly added, such as using Span 80 (S80) in combination with Tween 80 (T80). In addition, the products' taste plays an important role in consumers' acceptance and needs to be considered in the development of emulsion beverages. Currently, natural sweeteners such as stevia extract are widely used as a sugar substitute in the beverage industry in response to health-conscious consumers. However, apart from sweetening, these sweeteners can influence a product's properties such as color, flavor, and texture, thus appropriate types and concentrations of sweeteners need to be evaluated for each product.

There are research studies on the development of beverages made from rice bran oil and their biochemical effect in laboratory animals. The research suggested that emulsion drinks from rice bran oil helped increasing HDL cholesterol levels and significantly reduced blood pressure levels in experimental animals [6]. Moreover, there is a report suggesting that rice bran oil emulsion helped preventing metabolic syndrome and reducing inflammation which is linked to chronic diseases, such as diabetes, coronary artery disease, etc. [7].

From searching for information, there is no report on the use of Nongkhai black jasmine rice bran oil, which is indigenous colored rice for use in functional beverage products. Therefore, this research aims at studying the chemical and biological properties of Nongkhai black jasmine rice bran oil and studying the utilization of rice bran oil to develop an emulsified functional drink product. This could promote the utilization and add value to Nongkhai black jasmine rice bran, as well as, help reducing environmental problems arising from waste materials and lead to sustainability.

2 Materials and methods

2.1 Materials

The Nongkhai black jasmine rice paddy was obtained from a community enterprise in Sakonnakhon province, Thailand. All reagents were of analytical grade. Double-distilled and deionized water was used in the preparation of all solutions. Ingredients for emulsion formulation including erythritol mixed with stevia extract sweetener (Equal®), polysorbate 80 (Tween80), and sorbitan oleate (Span 80) were acquired from a local market in Prachinburi province, Thailand.

2.2 Rice bran preparation and oil extraction

After milling, rice bran was immediately heated at 110 ºC in the hot air oven for 10 min in order to inactivate lipase activity. Then, the rice bran was sealed in a plastic bag and kept at -20 ºC for further extraction.

Rice bran oil was extracted using hexane as a solvent by Soxhlet extraction method for 6 h. Hexane was removed by using a rotary evaporator at 175 mbar, 45 ºC. Then, the oil was heated in a hot air oven at 70 ºC to assure that there was no hexane left in the oil. The oil yield was determined by using the following equation;

$$Yield\ (\%) = \frac{Extracted\ oil\ weight\ (g)}{Rice\ bran\ weight\ (g)} \times 100 \quad (1)$$

2.3 Preparation of rice bran oil-in-water emulsion drink

The oil-in-water emulsion was developed by dispersing 1% (w/w) of Nongkhai black jasmine rice bran oil with 0.1% (w/w) of Tween80/Span80 (1:1 w/w) as emulsifiers in water. The mixture was emulsified using a high shear homogenizer (IKA, T-25 basic Ultra-Turrax, Germany) at 16,000 rpm for 2 min followed by a 2-stage homogenizer at 1,000 bars 3 times. After that, all the samples were pasteurized in a water bath at 80 ºC for 10 min. The sweetness of the emulsion beverage was adjusted by adding erythritol mixed with stevia extract sweetener at 0.1, 0.2, 0.3, and 0.4 % (w/v) during the pasteurization. The emulsion samples were then packed in amber glass bottles and stored at 10 ± 2 ºC for further studies.

2.4 Determination of total phenolic content

The total phenolic content (TPC) of samples was analyzed using Folin Ciocalteu assay [8]. Briefly, 20 µL of the sample was mixed with 100 µL of Folin-Ciocalteu reagent and 80 µL of 20% sodium carbonate. The mixture was incubated for 30 min in the dark at ambient temperature. Then, the absorbance of the samples was determined at 765 nm using a microplate reader (BMG Labtech, SPECTRO star Nano, Germany). The TPC was calculated from the calibration curve of gallic acid (20-100 µg/mL) (R^2 = 0.9909). The results were expressed in µg gallic acid equivalent (GAE)/g oil.

2.5 Determination of total flavonoid content

The total flavonoid contents (TFC) of samples were determined according to aluminum chloride colorimetry assay [9]. Briefly, sample (50 µL) was mixed with 10% aluminum chloride (10 µL), 1 M potassium acetate (50 µL) and 95% ethanol (150 µL). After incubation for 40 min, the absorbance of the samples was determined at 415 nm using a microplate reader (BMG Labtech, SPECTRO star Nano, Germany). The TFC was calculated from the calibration curve of quercetin (20-100 µg /mL) (R^2 = 0.9992) and expressed in µg quercetin equivalent (QE)/ g oil.

2.6 Determination of γ-oryzanol

The γ-oryzanol content was determined using a spectrophotometric method according to the CODEX-STAN 210-1999 method [10]. Sample was dissolved in isopropanol (100 mg/mL) and measured the absorbance at 315 nm using a microplate reader (BMG Labtech, SPECTRO star Nano, Germany). The γ-oryzanol content

was calculated from a standard curve of γ-oryzanol (10-50 µg/mL) (R^2 = 0.9994) and expressed in µg/ g oil.

2.7 Determination of antioxidant activity

Antioxidant activities of the samples were investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2'azinobis (3-ethylbenzothaiazoline-6-sulfonic acid (ABTS) cation radical scavenging assay, and ferric reducing antioxidant power assay (FRAP). Brief details for each assay were described as follows.

According to the DPPH assay, DPPH ethanolic solution (100 µL) was mixed with 20 µL of a sample or standard solution and kept in the dark for 30 min [11]. The absorbance was recorded at 517 nm using a microplate reader (BMG Labtech, SPECTRO star Nano, Germany). The DPPH radical scavenging activity was calculated from the calibration curve of trolox (20-200 µg/mL) (R^2 = 0.9907). The results were expressed in µg trolox equivalent (TE)/ g oil.

ABTS assay was performed by following the method of Arnao *et.al*., (2001) [12]. The ABTS radical solution composing of 4.9 mM ABTS and 14 mM $K_2S_2O_8$ at a ratio of 1:1 (v/v) was prepared and left for 24 h prior use. The ABTS radical solution was diluted with ethanol to achieve an absorbance of 0.7 ± 0.1. The sample (5 µL) was mixed with ABTS radical cation solution (500 µL) and incubated for 10 min. The absorbance was determined at 734 nm using a microplate reader (BMG Labtech, SPECTRO star Nano, Germany). The antioxidant activity was calculated from the calibration curve of trolox (200-1000 µg/mL) (R^2 = 0.9913) and expressed in µg trolox equivalent (TE)/ g oil.

For the FRAP assay, the FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl) and 20 mM ferric chloride solution in the proportions of 1:1:10 (v/v), respectively [13]. The sample (50 µL) was mixed with the FRAP reagent (150 µL) and incubated for 10 min. The absorbance at 595 nm was recorded using a microplate reader (BMG Labtech, SPECTRO star Nano, Germany). The antioxidant activity was calculated from the Trolox standard curve (0-50 µg /mL) (R^2 = 0.9957) and expressed in µg trolox equivalent (TE)/g oil.

2.8 Sensory analysis of rice bran oil-in-water emulsion drink

The oil-in-water emulsion drinks were subjected to Just-about-right and 9-point hedonic sensory test by 30 untrained panelists. Testing was done in a sensory laboratory where each panelist was randomly served 4 emulsion drinks (30 mL in a plastic cup) in cold (10 ± 1°C). An unsalted biscuit was used to cleanse the palate between each sample test. Overall liking score was assessed on a 9-point hedonic scale, where 1 = 'dislike extremely' and 9 = 'like extremely'. At the same time, the 5-point just-about-right (JAR) scale was used to determine the appropriateness of sweet intensity of the samples, where 1 = 'Not nearly sweet enough'; 2 = 'somewhat not sweet enough'; 3 = 'just about right'; 4 = 'somewhat too sweet'; 5 = 'much too sweet'.

2.9 Statistical analysis

All data shown in this study represent the mean values ± standard deviation of triplicate measurements. The statistical differences between mean values were analyzed by analysis of variance (ANOVA) using IBM SPSS Statisics 25.0 and compared using Duncan's multiple-range test with a significance level of 0.05.

3 Results and discussion

3.1 % Yield and bioactive compound contents of Nongkhai black jasmine rice bran oil

Nongkhai black jasmine rice bran was extracted by Soxhlet apparatus using hexane as a solvent. Hexane is commonly used in the vegetable oil production industry because of its low polarity and high productivity. Moreover, the solvent extraction process is simple to manage. In this study, the yield of oil from Nongkhai black jasmine rice bran was 12.38%. It's been previously reported that oil yield from other rice cultivars was between 13-22% [14,15]. The yield of rice bran oil depends on the type of solvent, method, extraction time and temperature as well as the varieties of rice used as raw materials.

From Table 1, bioactive compound content of Nongkhai black jasmine rice bran oil was investigated. Among other components, phenolic compounds are substances with antioxidant activity found in rice bran. The major groups of phenolic compounds are phenolic acids (e.g. p-coumaric acid, ferulic acid, gallic acid, protocatechuic acid, and sinapic acid) and flavonoids (quercetin, catechin, rutin, and apigenin). The total phenolic and total flavonoid content of Nongkhai black jasmine rice bran oil were 120.03 ± 1.26 µg GAE/g oil and 46.90 ± 0.69 µg QE/g oil, respectively. Janu and co-workers (2014) revealed that the total phenolic contents of some common vegetable oils were in the range of 0.33-3.09 mg GAE /100 g oil (approximately 3-30 µg GAE /g oil) [16]. In terms of total flavonoid content, Ghasemzadeh *et. al.,* (2018) reported that the total flavonoid contents of Malinin, Sangyod, and Leum Phua black glutinous rice bran were 0.52-0.57 mg QE/g oil (about 520-570 µg QE/g oil) [17].

Table 1. Bioactive compound content and antioxidant activity of Nongkhai black jasmine rice bran oil

Chemical properties	Mean±SD
Total phenolic content (µg GAE/g oil)	120.03 ± 1.26
Flavonoid content (µg QE/g oil)	46.90 ± 0.69
γ-Oryzanol content (µg/g oil)	236.73 ± 7.54
Antioxidant activities	
DPPH assay (µg TE/ g oil)	1676.63 ± 76.86
ABTS assay (µg TE/ g oil)	592.75 ± 46.22
FRAP assay (µg TE/ g oil)	1.31 ± 0.22

γ-oryzanol is a group of ferulate compounds mainly found in rice bran oil. It possesses health benefits as there have been evidences that could help reducing the risk of cardiovascular disease and lowering the levels of low-density lipoprotein cholesterol [18,19]. Thus, it is well known as one of the heart-friendly oils. In this study, the γ-oryzanol content of Nongkhai black jasmine rice bran oil was 236.73 ± 7.54 μg/g oil (Table 1). This is relatively low compared to other Thai rice bran oils of which are reported to contain γ-oryzanols ranging from 1.75-17.54 mg/g oil (1750-17500 μg/g oil). The variation of γ-oryzanols depends on factors such as the extraction technique, rice cultivars, and farming region, etc. [20].

Regarding to antioxidant activity of the extracted oil, different assays were performed including DPPH, ABTS, and FRAP assays. We found that the antioxidant activity of the Nongkhai black jasmine rice bran oil tested by DPPH, ABTS, and FRAP were 1676.63 ± 76.86, 592.75 ± 46.22, 1.31 ± 0.22 μg TE/g oil, respectively (Table 1). It was observed that the antioxidant activities of the extracted oil varied among different assays, which could be a result of the systems and reaction processes occurred in different methods. Thus, it is important to apply different techniques to assess the antioxidant activity of tested samples in order to ensure the accuracy and thoroughness of the findings. A previous study reported that other Thai pigmented rice bran oils possessed the DPPH radical scavenging activity of 12.19-18.80 mg TE/g oil and ABTS radical scavenging activity of 13.05-17.17 mg TE/g oil [20]. Significant variations in the antioxidant capacity of rice bran oil from various cultivars indicated that the phytochemical composition and antioxidant capacity of rice varieties depend on a variety of factors, especially cultivars and extraction techniques. The antioxidant activity of rice bran oil could be owing to lipophilic antioxidants such as tocopherol (vitamin E) or γ-oryzanol, etc.

3.2 Development of rice bran oil-in-water emulsion drink

Due to its unique qualities and nutritional values, rice bran oil has found many uses as a functional oil in food industry and pharmaceuticals. In the present study, the extracted Nongkhai black jasmine rice bran oil was formulated into a functional oil-in-water emulsion beverage. The appearance of emulsion was white and cloudy without creaming separation. The erythritol mixed with stevia extract sweetener was added in the emulsion beverage at 0.1, 0.2, 0.3 and 0.4% (w/w). The hedonic scale was used in combination with just-about-right test to measure the optimum intensity of sweetness of an emulsion beverage. The hedonic score was shown in Table 2. The emulsion beverage with the addition of 0.4% sweetener obtained the significantly highest hedonic score in terms of taste and overall liking. The taste and overall liking score of 6.8 ± 1.1 and 7.0 ± 1.1, respectively, which represent moderate liking.

Table 2. The 9-point hedonic score of Nongkhai black jasmine rice bran oil -in-water emulsion beverage with different concentrations of sweetener

% Sweetener	Mean ± SD	
	Taste	Overall liking
0.1	4.6 ± 1.6^{b}	4.7 ± 1.6^{c}
0.2	4.9 ± 1.4^{b}	5.0 ± 1.3^{bc}
0.3	5.3 ± 1.4^{b}	5.5 ± 1.3^{b}
0.4	6.8 ± 1.1^{a}	7.0 ± 1.1^{a}

Note: Means with different letters within a column are significantly different ($p \leq 0.05$).

The 5-point just about right (JAR) test was performed along with the hedonic test in order to assess whether the sweetness level is appropriate. The results of the JAR evaluation are shown in Table 3. The 96.7% of panellists, which is more than 70% rated the samples with 0.4% sweetener as being the just-about-right level for sweetness. Moreover, the % net effect was less than 10%. This suggests that the addition of 0.4% sweetener into the rice bran oil emulsion was the most appropriate compared with other concentrations in this study.

Table 3. Percentage of panelists based on the 5- point just about right test of Nongkhai black jasmine rice bran oil -in-water emulsion beverage with different concentrations of sweetener

Sweetener	Just about right scale)%(					Net effect
(%)	1	2	3	4	5	(%)
0.1	46.7	40.0	3.3	6.7	3.3	76.7
0.2	33.3	53.3	3.3	6.7	3.3	76.7
0.3	16.7	56.7	10.0	13.3	3.3	56.7
0.4	0.0	3.3	96.7	0.0	0.0	3.3

Note: 1 = Not nearly sweet enough; 2= Somewhat not sweet enough; 3= Just about right ; 4 = Somewhat too sweet; 5= Too much sweet

The Nongkhai black jasmine rice bran oil-in-water emulsion beverage with 0.4% (w/w) sweetener was chosen according to the sensory evaluation and was tested for the bioactive compound content and antioxidant activity as shown in Table 4. The Nongkhai black jasmine rice bran oil-in-water emulsion beverage contained γ-oryzanol of 13.3 ± 0.3 μg/mL and possessed ABTS radical scavenging activity of 267.0 ± 37.7 μg TE/mL.

Table 4. Bioactive compound content and antioxidant activity of Nongkhai black jasmine rice bran oil-in-water emulsion beverage with 0.4% (w/w) sweetener

Chemical properties	Mean±SD
Total phenolic content (μg GAE/mL)	n.d.
Flavonoid content (μg QE/mL)	n.d.
γ-Oryzanol content (μg/mL)	13.3 ± 0.3
Antioxidant activities	
DPPH assay (μg TE/mL)	n.d.
ABTS assay (μg TE/mL)	267.0 ± 37.7
FRAP assay (μg TE/mL)	n.d.

Note: n.d. means not detected.

However, some bioactive compounds including phenolic and flavonoid could be degraded during the process of emulsification and pasteurization which occurred at high pressure and temperature. Nayak *et al.*,

(2020) reported that total phenolic contents, total flavonoid contents, antioxidant activity of elephant apple juice were reduced as affected by pasteurization process [21]. Moreover, the oil content used in this study was low (1% w/w) leading to low content of bioactive compounds. To increase the antioxidant activity of the emulsion beverage, a higher oil content could be added.

4 Conclusions

This study reports the bioactive compound content and the antioxidant activity of Nongkhai black jasmine rice bran oil. The oil contained a significant amount of phenolic 120.03 ± 1.26 μg GAE/g oil, flavonoid 46.90 ± 0.69 μg QE/g oil and γ-oryzanol 236.73 ± 7.54 μg/g oil. The antioxidant activity of the oil tested by DPPH, ABTS, and FRAP were 1676.63 ± 76.86, 592.75 ± 46.22, 1.31 ± 0.22 μg Trolox equivalent/g oil, respectively. The Nongkhai black jasmine rice bran oil - in-water emulsion beverage was successfully developed. The emulsion containing 0.4 % sweetener had the highest overall liking score of 7.0 ± 1.1, representing moderate liking. The emulsion beverage contained γ-oryzanol as a lipophilic bioactive component with the antioxidant activity tested by ABTS of 267.0 ± 37.7 μg Trolox equivalent/mL. This study suggests that Nongkhai black jasmine rice bran oil possesses beneficial components including γ-oryzanol as well as other lipophilic antioxidants such as tocopherol. Hence, it could be used in food products and nutraceuticals for sustainable development.

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