Development of functional beverage with rice bran protein (Hom mali 105)

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Abstract. In this research, rice bran protein extracted was added into food model such as fruit juices, and fruit-flavored beverages that are consumed for refreshment, quenching thirst, and nutritional value to make consumers refreshed, quench and provide nutritional value based on important substances added to functional beverages. The development of ready-to-drink fruit juice offers consumers convenience and creates new experiences from functional beverages that were available in the market. The value-added of rice bran by extract the protein and extend its application in food. The enzymetic method was used to extract protein from rice bran (Hom mali 105). Physical and functional properties of rice bran protein extracted had 0.45 g/ml of density, 7.73% of moisture content, 22.32% of protein and 7.28 mg gallic/g for phenolic content and 2,495.26 mg ASC/g sample of ABTS values. The development of functional fruit juice consisted of two concentrate fruit juice (80 red grapes : 20 apples ratio), the mixture was mixed with 60% fruit juice, 30% water, 8% syrup and 2% rice bran protein powder, which was pasteurized under 75 °C for 2 minutes 50 s. Microbial analysis showed that the values met the standard (microbial count less than 250 colonies/ml, Coliform bacteria and Escherichia coli less than <1.1 MPN/100 g). Sensory evaluation of color, flavor, sourness, sweetness, and overall acceptance (n=30) was conducted. The assessment scores for the various attributes were moderately favorable. 91% of consumers accepted of the product. The extracted protein with functional beverage was offers a new alternative for consumers who want to increase the nutritional value in a beverage and diversify the a new alternative beverage product.

1 Introduction

The growth of plant protein market is increasings, plant based market is projected to increase from year 2018 to 2030 4.6 billion to 85 billion and year 2026 reach 30.9 billion [1, 2]. Therefore, the demand for inexpensive protein sources may increase, and this utilization increases the value of the bran from rice milling industry. The extraction of rice bran protein from rice milling is the most cost-effective and optimal way to use the milling waste. It has been reported that up to 12-15% of rice bran protein is considered a potential source of high-quality protein and other important nutrients such as vitamins, minerals, and fiber, as well as albumin and globulin [3, 4]. Rice has protein as its second dominant (6-7%) major nutrient. rice protein has four fractions (albumins, globulins, prolamins and others) [5].

Rice bran protein is a source of high-quality hypoallergenic protein, commonly used as an ingredient in the food of infants and children product [6]. In addition, It can indicates that the amino acid composition of rice bran protein is similar nutritious to casein protein and soy protein isolate. It is possible to extract proteins from rice bran as a source of essential amino acids, and the extracted protein was analyzed for the number and profile of essential amino acids. They can be classified as follows: lysine and tyrosine were high, phenylalanine and tryptophan were moderate [6]. The quality of the amino acids also depends on the storage and methods of extracting protein from rice bran as well.

Thailand is the fourth largest rice exporter in the world in 2021 [7]. The production volume of rice in Thailand was 31.5 million tons in 2021/2022 [7,8]. Of all the rice varieties, the majority are white rice, Hom mali 105 rice and sticky rice accounting for 81% from total productivity of thai rice during years 2021/2022 [9]. Thai's population mainly consumes rice that makes the amount of rice bran after milling more than 2.5 million tons per year, which is interesting for utilization rice bran and protein extraction. Hom mali in Thai, the name is often thought to imply to "Jasmine rice" which is considered one of the world's most dilicious cultivar of rice.

At present, the market for fruit juice in term of bottled beverages has a great opportunity to expand. The global fruit juice market size reached US$ 153.8 billion

in 2023, exhibiting a growth rate (CAGR) of 3.8% during 2024-2032 [10]. Consumer are concern in health, seeking in new fruit juice and exciting taste experience and they are also willing to spend on healthier beverage options, including high quality fruit juice with a new flavors and nutrition ingredients [11]. Therefore, the idea of this experiment was to add rice bran protein concentrate or plant-based protein to liquid food products such as fruit juices or fruit-flavored beverages to offer refreshment, quench thirst, and provide the nutritional value of a functional beverage. This gives consumers a convenient alternative to other functional beverages that are available on the general market.

This research started with an analysis of the chemical composition of jasmine rice bran (Hom mali 105). Protein was extracted from rice bran by a commercial protease enzyme, concentrated by vacuum evaporation, and then freeze-dried into powder form. Concentrated plant-based protein from rice powder was analyzed for the physicochemical and functional properties of the extracted proteins, including antioxidant activity, solubility, emulsifying and foaming properties. Moreover, the idea of this experiment was to add rice bran protein concentrate or plant-based protein to fruit juices as a food model.

This research aimed to 1) use plant-based protein from rice by developing it as a functional beverage product; 2) develop the beverage formula at different pasteurization conditions and 3) study the physical, chemical, microbial properties, and acceptance of the developed products.

2 Materials and methods

2.1 Materials

Defatted rice bran, Hom mali105 was obtained from Suan Dusit Rajabhat, Rice mill factory Co Ltd. (Prachinburi province, Thailand). The defatted rice bran was packed under low density polyethylene (LDPE) vacuum plastic bag and stored under -18℃ in the fridge.

2.2 Protein extraction

Rice bran digestion using the enzymes technique. For the first digestion used alcalase by added rice bran (without fat) at the ratio of rice bran:buffer solution pH8 19.23g:250ml and 0.01% of alcalase [3]. Shaking the solution for 30 min at 50℃ during extracted, then inhibited the enzyme reaction at 85℃ for 10 min. For the second digestion used flavourzyme; 0.075% flavourzyme was added into rice bran solution. The pH of the solution was adjusted to 6 and extracted for 4 h at 50℃, then inhibited the enzyme reaction at 85℃ for 10 min. The extracted rice protein was filtered by a vacuum filter, then evaporated for 70℃ 120 mbar by a rotary evaporator (R-205, Buchi, Switzerland). Rice bran protein concentrated was dried using freeze-dryer (Alpha1-4 LSC, Christ, Germany). An operating condition for freeze dry were -40℃ for 4 h. for pre-freezing step then -50℃ for 24 h for main-freezing step and -5℃ for 5 h for final dry step. The concentrated rice bran protein was in the powder form and kept in aluminium foil bag and stored in the fridge.

2.3 Materials

Bulk density was determined using graduated measuring cylinders and calculated as weight per unit volume (g/ml) [12]. Colors were determined using a Hunter LAB colorimeter, analyzed by the CIE L*, a* and b* system. Moisture content was measured according to the standard method of AOAC [13] and water activity (a_w) were measured with an Aqua lab meter. The degree of hydrolysis analysis (%) was measured by reacting with trinitrobenzenesulfonic acid (TNBS reaction), according to Adler-Nissen J. [14, 15]. The degree of hydrolysis (%) was calculated as follows:

DH (%) = (Number of peptide bonds cleaved/Total number of peptide bonds in sample) x100

Protein characteristics and functional properties:

Functional properties of rice protein extract, phenolic content and antioxidant activity;

Total phenolic analysis was performed according to the method [16] and calculated from a standard curve of gallic acid in range of 0-10 mg/100 ml.

Antioxidant capacity; The DPPH method was applied by mixing rice protein extracts with DPPH reagent to determine their efficacy in scavenging free radicals. The absorbance was measured at a wavelength of 571 nm [17] and calculated using the following equation:

DPPH (%) = [(absorbance of DPPH – absorbance of sample)/absorbance of DPPH] x100

"Ferrous Ion-chelating: FIC assay" was measured followed the method of [18] and calculated the percentage of inhibition:

Inhibition (%) = [1- (Asample1 - Acontrolblank1) /(Asample0 – Acontrolblank0)]x100

The "ABTS radical scavenging capacity assay" was measured as the method of Floegel, A. [19] and the inhibition capacity free radicals was calculated;

Inhibition (%) = [(A0 - A1)/A0] x 100

Where A0 is the ABTS absorbance, A1 is the sample + ABTS absorbance

Emulsifying properties were reported as emulsion activity index (EAI) and emulsion stability (ES) which were measured according to Pearce and Kinsella [20].

2.4 Formulation of fruit juice with Rice bran protein extracted

A formula was developed for fruit juice with rice bran protein extracted that contained high-functional properties. The prototype of fruit juice was shown in Table 1.

Table 1. Basic formula of fruit juice

Materials	Percentage (%)
Concentrated fruit juice	60.0
Water	30.0
Syrup	8.0
Rice bran protein extracted	2.0

Source: Modified from School of Culinary Arts

Preheated the rice bran protein in water and then added the concentrateded fruit juice, water and syrup together. Stirred and combined all materials for 3 minutes until the fruit juice well mixed after that fruit juice has to be starting the heating process, the conditions was showed in Table 2.

Table 2. Pasteurization conditions for fruit juice

Treatment	Temperature (℃)	Time
1	60	5 minutes
2	70	3 minutes
3	75	2 minutes 30 seconds
4	80	50 seconds

Source: FDA Recommended temperatures and time's pasteurization of apple juice product, pH<4.0 or below.

The optimum formula for fruit juice was used as the finished product prior to the consumer acceptance test to produce the formula as an acceptable functional drink. Subsequently, all aspects of quality including chemical properties, physical properties, and sensory evaluation were evaluated.

2.4.1 Chemical analysis

Protein content of product was conducted in accordance with the methods of AOAC [13]. Total acid content was measured under the method of A. Woo. [21] and calculated as ascobic acid in sample (mg/ml sample).

2.4.2 Physical Properties

The color of product was measured using a colorimeter (CR-410, Konica Minolta, Inc., Japan) by the CIE system (L*, a*, b*) and reported as average individual values marked as L* (lightness), a* (+a = red, -a = green), and b* (+b = yellow, -b = blue).

2.4.3 Sensory analysis

The preference test of fruit juice with rice bran protein in terms of color flavor sweetness sourness and overall liking was performed by 30 untrained panelists. 10 ml of fruit juice samples were packed into plastic cup covered with a plastic lid and subjected to panelists for evaluation. Code each sample with random number and subjected four sample per panelist. The 9-hedonic scale was used that included 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike, 5 = neither like nor dislike, 6 = like, 7= like moderately, 8 = like very much and 9 = like extremely [21].

2.5 Acceptability test

For a survey focusing on 100 target consumers—including age between 18-60 year old in four area (Prachinburi, Nakhon Nayok, Pathum thani and Bangkok). A questionnaire was utilized to gather data. The questionnaire asked questions on demographics, product preferences rated by 9-point hedonic scale, and consumer acceptance as determined by binomial (yes/no) scale.

2.6 Microbiological test

Microbial analysis: Total plate count (CFU/ml), Coliform bacteria (MPN/100ml) and *Escherichia coli* (MPN/100ml) were investigated according to FDA-Bam, 2011. Fruit juice with protein extracted 50 ml was blended with 450 ml of sterilized saline 0.85% using a stomacher. Subsequently decimal dilution series (10-fold) were prepared from the primary dilution using peptone solution. Pour-plate method using plate count agar (PCA) media (Merck, Germany) was used for total plate count and incubated at 37ºC for 48 h. Most probable number (MPN) method was used for coliform bacteria determination. The inoculated LST/BGLB broth were incubated at 35ºC for 48 h. For *Escherichia coli*. determination, the inoculated LST broth were incubated at 44.5ºC for 24 h. All analyses were performed in triplicate.

2.7 Statistical analysis

Formulation data were calculated in triplicate as the mean with a standard deviation. The results for process development were conducted based on a Completely Randomized Design (CRD). Data were subjected to SPSS software version 26. Mean values were compared using Duncan's Multiple Range Test after one-way ANOVA. The frequency (%) and mean liking score of consumers on the developed fruit juice represented the acceptance test results. Statistical difference was established at $p < 0.05$.

3 Results and discussion

3.1 Physical and functional properties of rice bran protein

The color value of rice bran protein extracted was luminance (L*) of 85.55, redness (a*) of 1.98, yellowness (b*) of 15.98; water activity was 0.29; density was 0.45 g/ml; moisture content was 7.73%; nitrogen and protein content were 8.26% and 22.32%, respectively. The degree of hydrolysis was 19.42% which contribute to the enzyme hydrolyzed peptide bounds in rice bran. Then the concentration of amine group was increase from the protein hydrolysates [22]. Sari, Y.W. reported that the conditions of enzymetic method (more concentration and time) to decompose increasing degree of hydrolysis (%). Moreover, for the other extraction method such as microwave technique showed the lightness value of rice bran protein was dark-

reddish than enzymetic method [23]. Whereas, bulk density of rice bran protein extracted was 0.3 g/ml after using microwave technique [24].

As shown in Table 3, the total phenolic content (mg gallic/g sample) of the rice protein powder was 7.28. Antioxidant activities were DPPH scavenging activity (%); FIC (EDTA eq) and ABTS (mg ASC/g sample) of rice bran extract proteins were 4.04, 0.40, and 2495.26, respectively. Emulsifier properties reported as EAI (m^2/g protein) and ESI (min) were 8.78 and 12.23, respectively. The DPPH method measure of free radical scavenging capacity which antioxidant act to inhibit lipid oxidation. Whereas FIC method has the ability to chelate transition metal ions (Fe^{2+} $Cu2^{+}$) which are act as pro-oxidant and induce oxidative stress in food system. For ABTS method is a measure of antioxidats to scavenge free radical and the ABTS is carry out in aqueous system. If system high in hydrophilic antioxidant was better reacted by ABTS [18, 19]. From the result revealed that in food system using water as the medium, the use of rice bran protein extracted has high hydrophillic antioxidant properties.

The foaming capacity of the rice protein extracts showed that at 1 minute the foaming capacity was 80.19%; however, after 5 minutes the foaming capacity was reduced to 58.09%. The formation of bubbles will change after churning and shaking. Factors affecting the collapse of the bubbles were film thickness, the surface tension between the junction of air-liquid interfaces, and the elasticity of the protein [25].

In addition, the foam stability was influenced by the rheological properties of the film and the charge density on the protein molecule, which was related to the percentage of hydrolysis of the protein [26] . It has been reported that when the percentage of hydrolysis increases, the foaming and solubility also increased which, suitable for use in beverage products or water-based products for foam stability which was a measurement of the volume of bubbles overtime after spinning. The results of the solubility analysis (%) shown that the solubility depended on the pH. When the pH increases, the solubility increases. The pH was 8, resulting in solubility of 46.61. In protein system, long chain peptides protein have lower solubility than short chain peptides, which is related to zwitterion form of amino acid in protein [27].

3.2 Qualities of fruit juice with Rice bran protein extracted

The total acid content of fruit juice with rice bran protein extracted was showed in Table 4. The result showed that at low temperature long time pasteurization had lowest total acid content while at high temperature short time pasteurization had highest total acid content. The total acid is the total amount of all the available hydrogen ions in a solution [28]. The total acid of fruit juice is very much affected by the method and condition of pasteurization. It also changes during the storage and condition of the storage [29]. Therefore, long time duration in pasteurization conditions affected on the loss of hydrogen bound to organic acid in fruit. In table 5, the total soluble solid, fruit juice with rice bran protein extracted had the range of 16.93-17.07, while pH values of fruit juice were 3.57-3.66.

The Color of fruit juice with rice bran protein extracted (Table 6) indicated that due to the pasteurization conditions from low temperature long time (60℃ 5minutes) to high temperature short time (80℃ 50seconds), adding the stresses conditions decreased the lightness value (L*) while the red value (a*) and yellow value (b*) of fruit juice was not significant different ($p \leq 0.05$). However, because the grape juice would given the intensity of the red tone, so the red value of fruit juice with rice bran protein extracted did not changed.

Table 3. Functional properties of rice bran protein

	Functional properties of rice bran protein
1) Phenolic content (mg gallic/ g sample)	7.28±0.08
2) DPPH scavenging activity (%)	4.04±1.03
3) FIC (EDTA eq.)	0.40±0.007
4) ABTS (mg ASC/ g sample)	2495.26±287.10
5) Emulsifying properties	
EAI (m^2/g protein)	8.78±0.70
ES (minutes)	12.23±0.27
6) Foam capacity (%)	
0 minutes	100
1 minutes	80.19±0.51
5 minutes	58.09±8.22
15 minutes	28.59±2.81
7) Foam stability (FS)	0.73±0.08
8) Solubility (%)	
pH 4.0	29.34±1.28
pH 6.0	42.84±0.57
PH 8.0	46.61±1.04

Table 4. Total acid content of fruit juice with rice bran protein at different pasteurization conditions

Formula	Total acid content (mg/ml)
1. 60 °C 5 minutes	0.43±0.04 ^{b}
2. 70 °C 3 minutes	0.50±0.00 ^{ab}
3. 75 °C 2 min. 30s.	0.53±0.04 ^{a}
4. 85 °C 50 s.	0.50±0.00 ^{ab}

Note: Means in column followed by different letters represent significant differences ($p \leq 0.05$).

Table 5. Total soluble solid content and pH of fruit juice with rice bran protein extracted at different pasteurization conditions

Formula	TSS ^{ns}	pH
1	17.07±0.12	3.52±0.02 ^{c}
2	16.93±0.12	3.61±0.01 ^{b}
3	17.07±0.12	3.75±0.01 ^{a}
4	17.00±0.20	3.76±0.00 ^{a}

Note: Means in column followed by different letters represent significant differences ($p \leq 0.05$). ^{ns} letters represent not significant difference and ** Formula 1-4 different in pasteurization conditions; 1) 60℃ 5minutes; 2) 70℃ 3minutes; 3) 75℃ 2minutes 3seconds; and 4) 80℃ 50 seconds.

Table 6. Color of fruit juice with rice bran protein at different pasteurization conditions

Formula**	L*	a*ns	b*ns
1	20.16±0.30[a]	16.84±0.85	-0.83±0.07
2	17.47±0.28[ab]	16.71±0.23	-1.30±0.23
3	18.63±0.58[ab]	16.15±0.23	-0.96±0.18
4	11.89±1.14[c]	16.37±0.86	-1.21±0.70

Note: Means in column followed by different letters represent significant differences ($p \leq 0.05$). ns letters represent not significant difference. ** Formula 1-4 different in pasteurization conditions; 1) 60℃ 5minutes; 2) 70℃ 3minutes; 3) 75℃ 2minutes 3seconds; and 4) 80℃ 50seconds.

Table 7 displays the sensory qualities of fruit juice with rice bran protein extracted at different pasteurization conditions. The mean liking score by the panelists measurement 9-point hedonic scale were not significantly different ($p>0.05$) in every sensory attribute including color, flavor, sweetness and overall linking, which were rated like to moderate like (6-7) while sourness was rated neither like nor dislike (5).

Table 8 shows the microbial analysis of fruit juice with rice bran protein at different pasteurization conditions. Results showed that fruit juice treated with low temperature long time (60℃ 5minutes) had the highest in total microbial, coliform bacteria and *E. coli.* Wherease fruit juice treated with higher temperature short time, the lower in amout of total microbial, coliform bacteria and *E. coli. w*ere report. The optimal pasteurization condition for fruit juice with rice bran protein should be suggest at 75℃ for 2 minutes 3 seconds, deu to the microbial and sensorial qualities which described by total microbial <250 colony/ml, coliform bacteria <1.1 MPN/100ml, *E. coli.<1.1* MPN/100ml in term of safety and the preference by hedonic score including color, flavor, sweetness and overall linking which were rated like to moderate like and it also overall liking attribute was highest liking score, respectively.

Table 7. Sensory analysis of fruit juice with rice bran protein at different pasteurization conditions

*	Color[ns]	Flavor[ns]	Sweetness[ns]	Sourness[ns]	Overall[ns]
1	6.96±0.98	6.80±1.09	6.16±1.08	5.16±1.08	6.23±1.04
2	5.96±1.30	6.46±1.35	6.03±1.16	5.66±1.41	6.18±1.11
3	6.43±1.06	6.33±1.56	6.56±1.33	5.83±1.09	6.60±1.20
4	6.40±1.15	6.16±1.26	6.33±1.06	6.23±1.02	6.46±1.13

Note: Means in column followed by same ns letters represent no significant differences ($p>0.05$). *Formula 1-4 different in pasteurization conditions; 1) 60℃ 5minutes; 2) 70℃ 3minutes; 3) 75℃ 2minutes 3seconds; and 4) 80℃ 50seconds.

Table 8. Microbial analysis of fruit juice with rice bran protein at different different pasteurization conditions.

Microbial counts	Formulas			
	1	2	3	4
Total microbial (colony/ml)	<300	<250	<250	<250
Coliform Bacteria (MPN/100ml)	3.6	<1.1	<1.1	<2.2
E. Coli (MPN/100ml)	3.6	<1.1	<1.1	<2.2

Note: Formula 1-4 different in pasteurization conditions; 1) 60℃ 5minutes; 2) 70℃ 3minutes; 3) 75℃ 2minutes 3seconds; and 4) 80℃ 50seconds.

3.3 Acceptability test

The results of the consumer's acceptance for fruit juice with rice bran protein are shown in Tables 9 to 11. This study includes demographic data, consumer liking and acceptability tests of 100 target consumers from Prachinburi, Nakonnayok, Pathum thani and Bangkok area. Sample was consumed by all respondents (100%)—55% women and 38% men—the majority of whom were age between 20-35 years old (52%), followed by the age of 36–49 years old (36%), and age older than 50 (10%). The highest percentage of consumer's education level was high school diploma level (55%). The students represented 53% of the respondents, followed by The government officers (19%). Over 10,000 THB was the average monthly income for 57% of the respondents, followed by 20,001–30,000 THB (31%).

Consumer's liking of the fruit juice with rice bran protein was evaluated on a 9-point hedonic scale for appearance, color, flavor, taste, and overall liking. The results indicate that consumer's liking of all characteristics was at the "like very much" level (7.1–7.4).

Consumer's acceptance and decisions to purchase the fruit juice with rice bran protein were surveyed, and the results showed that 9 1% of them accepted the product and 8 8% of them decided to buy the juice. It is feasible to continue developing it commercially according to the acceptance test results.

Table 9. Demographic data

Demographic attributes	Frequency (percentage)
1. Gender	
- Male	38.0
- Female	55.0
- Transgender	7.0
2. Age	
- less than 20 years old	2.0
- 20–35 years old	52.0
- 36–49 years old	36.0
- More than 50 years old	10.0
3. Education level	
- High school diploma	55.0
- Bachelor's degree	37.0
- Higher than bachelor's degree	8.0
4. Occupation	
- Students	53.0
- Government officers/employees	19.0
- Private company employees	8.0
- Personal business	17.0
- Housewives	3.0
5. Average monthly income	
- 10,000–20,000 THB	57.0
- 20,001–30,000 THB	31.0
- More than 30,000 THB	12.0

Table 10. Mean liking scores of fruit juice with rice bran protein (n=100)

Sensory attributes	Mean liking scores
Appearance	7.2±0.9
Color	7.4±1.1
Flavor	7.4±0.8
Taste	7.1±1.0
Overall liking	7.3±1.0

Table 11. Consumer's acceptance on fruit juice with rice bran protein (n=100)

Data	Frequency (percentage)
Consumer's acceptance on fruit juice with rice bran protein	
- Accept	91
- Not accept	9
Decisions on purchasing the fruit juice with rice bran protein extracted	
- Purchase	88
- Not purchase	12

4 Conclusion

Among the variations on the basic formula, the ratio of 60% fruit juice, 30% water, 8% syrup and 2% of rice bran proteion extracted was the food model for beverage. The sensory analysis on color, flavor, sourness, sweetness, and overall acceptance was combined with panelists (n=30), while the score were in range of like to moderatly like. From the study on the pasteurization conditions at 75 °C for 2 minutes 30 seconds was good for sensory and safety for microbial test. The microbial analysis found that the total microbial count was less than 250 colony/ml and > 1.1 of *Escherichia coli*, which met the Orange Juice Community Standard No. 275/2547 and the beverage was 91% accepted by consumers. Rice protein extracted functional beverage is a new alternative for consumers to increase nutritional value and add variety to beverage products.

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