Asia-Pacific Journal of Science and Technology: Volume: 27. Issue: 06. Article ID.: APST-27-06-19. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Technology Transfer Affairs Division,
Khon Kaen University, Thailand

Comparison of different protein extraction methods of rice bran protein concentrates

Farah N.A. Rahim[1], Wan Z.W. Ibadullah[1,*], Nazamid Saari[1], Nor A. Mustapha[2], Radhiah Shukri[2], Norhasnida Zawawi[1], Roselina Karim[2] and Ismail F.M. Rashedi[2]

[1]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor, Malaysia
[2]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor, Malaysia
[*]Corresponding author: wanzunairah@upm.edu.my

Received 7 July 2021
Revised 27 September 2021
Accepted 24 November 2021

Abstract

This study compared the protein content and yield of rice bran protein concentrates (RBPC) extracted via enzymatic and alkaline methods. Enzymatic extraction using xylanase produced RBPC containing 47.17% protein and a 19.80% protein yield. The optimal alkaline extraction conditions (pH 9, 30 °C) obtained 44.29% protein content and 21.80% protein yield. Further evaluation of alkaline extraction revealed that finer particles led to higher protein content (51.23%) and protein yield (26.85%) compared to 0.4 mm particles. Homogenization of the slurry or the addition of saline solutions did not significantly affect the protein content or yield. Rice bran protein extracted up to 72 h using 0.4 mm particle size gave the lowest protein content (5.62%) and protein yield (1.84%). The optimal alkaline extraction conditions (pH 9, 30 °C, 0.4 mm particle size, 1:4 solvent ratio, 15 min centrifuge, cloth filter, without overnight supernatant) for rice bran protein obtained extracts with a high protein content (43–51%) and yield (13–29%).

Keywords: Alkaline extraction, Enzymatic extraction, Homogenized alkaline extraction, Rice bran protein, Xylanase

1. Introduction

Rice is a daily staple food in Malaysia made from the processing of rough rice. Rice milling yields various by-products including rice hull (20%), rice bran, and germ (10%), with rice bran generally used as animal feed [1,2]. However, the plentiful supply of rice bran has promoted its utilization since rice bran is fortified with high-quality protein and is essential for food and pharmaceutical applications [2-5]. Moreover, rice bran has numerous health-promoting properties, such as being hypoallergenic, nutritious, and anti-cancer activity, with a desirable amino acid profile and high digestibility [6-8], thus is a sustainable protein source with high potential for application in the food industry, for example, the preparation of hypoallergenic infant formulation and gluten-free preparations. Furthermore, rice bran peptides have antioxidant, antihypertensive, anti-inflammatory, and anti-microbial properties [1].

The protein residue of rice bran is hardly extracted and insoluble in water because of its high glutelin content and strong aggregation properties through hydrophobic interactions and extensive bond cross-linking [9]. Various methods have been used to extract rice bran protein, such as enzymatic and alkaline extraction [1,3,8]. Enzymatic extraction yields protein at basic pH but is expensive, whereas alkaline extraction is the most common, simple, rapid, and low-cost method. However, the use of severe alkaline conditions (>pH 10) changes the nutritional characteristics of the protein, producing toxic products, denaturing, and hydrolyzing the protein, increasing the Maillard reaction, and alkaline-induced hydrolysis [3,5,6,8]. The extraction efficiency is also dependent on the extraction conditions such as agent concentration (alkali, enzyme), temperature, time, and the weight-volume ratio (extraction solvent: raw material) [10]. Therefore, this study compared the enzymatic and alkaline extraction of protein from defatted rice bran.

2. Materials and methods

2.1 Materials

Rice bran was purchased from rice mills in the Sekinchan area (Selangor, Malaysia) and xylanase 2753 enzyme from *Thermomyces lanuginosus* (minimum activity of 2500 unit/g) was purchased from Sigma. All chemicals and reagents used were analytical grade.

2.2 Defatted rice bran

Defatted rice bran (DRB) was prepared according to the method of Wang et al. [6]. Briefly, DRB was ground using a powder grinder (GW-FM/2HP, Taiwan) fitted with 0.40 mm wire mesh. Particles 0.177 mm in size (80 mesh, U.S. Standard sieve) (Laboratory Mill 120, Perten, Sweden) were used for enzymatic extraction and particle size analysis. The DRB was packed in polyethylene bags and stored at -40 °C before protein extraction.

2.3 Preparation of rice bran protein concentrate

2.3.1 Enzymatic extraction

Rice bran protein concentrate (RBPC) was extracted according to the method of Wang et al. [6]. DRB (10 g) was dispersed in deionized water (75 mL) and the pH was adjusted to pH 5 using 0.5 mol/L NaOH. The slurry was incubated with xylanase (240 xylanase unit/g bran) at 55 °C for 2 h to extract the protein. The enzyme activity was stopped by changing the pH to 9.5 with 0.05 mol/L NaOH and stirring for 30 min before centrifugation at 10,000 g for 20 min. The non-proteinaceous residue was vacuum filtered (filter paper no 4) and the supernatant was adjusted to pH 4.0 using 0.5 mol/L Hydrogen chloride (HCl). The protein was recovered by centrifugation t 10,000 g for 20 min, neutralized, frozen overnight, and freeze-dried. The RBPC was sealed in zip-lock PE bags and stored at 5 °C. The control rice bran protein was prepared under the same conditions using inactivated enzymes.

2.3.2 Alkaline extraction

The RBPC was prepared according to the method described by Gupta et al. [11] with slight modification. Briefly, 7.5 g of DRB was dispersed in 75 mL of distilled water and the pH was adjusted to 8, 9, or 10 using 0.5 mol/L NaOH. The slurry was stirred at 150 rpm in a shaking incubator for 1 h at 30 °C, 40 ºC, or 50 °C then centrifuged at 10,000 g for 30 min at 23 °C (Sigma 3-18K Sartorius, Germany). The slurry was vacuum filtered (filter paper no 4) to remove the insoluble protein residue and the supernatant was adjusted to pH 4.5 using 4 mol/L HCl and left overnight at 5 °C to precipitate the proteins. The slurry was separated by centrifugation at 10,000 g for 20 min at 4 °C and the precipitates were washed with distilled water, neutralized, then frozen overnight before freeze-drying (Scanvac Coolsafer 110-4, Labogene Aps, Denmark) for 96 h. The RBPC was sealed in zip-lock PE bags and stored at 5 °C.

The particle size (0.4 vs 0.177 mm), solvent ratio (1:4, 1:6, or 1:10 w/v), centrifuge time (15 min vs 30 min), filter cloth usage, and isoelectric point (pH 4.5) were compared at the standard extraction time of 1 h, 30 °C, and pH 9 using 0.5 mol/L NaOH and 4 mol/L HCl**.**

The extraction was also performed for 72 h before centrifugation for 15 min at 10,000 g, then the slurry extract was separated by a cloth filter, and the pH was adjusted for protein precipitation at the isoelectric point (pH 4.5) without being left overnight. The extract was centrifuged (15 min), washed, neutralized, frozen overnight, and freeze-dried before storage in zip-lock PE bags at 5 °C.

A single and double extraction were also compared. The DRB was dispersed (1:4 w/v solvent ratio), shaken (1, 2, 3 h), and centrifuged for 15 min at 10,000 g. The pH of the supernatant was adjusted to the isoelectric point (pH 4.5), then centrifuged (15 min), washed, neutralized, frozen overnight, and freeze-dried for 96 h. The RBPC was stored in zip-lock PE bags at 5 °C. For the double extraction, the insoluble residue was subjected to a second extraction to increase the protein residue. The insoluble residue was re-dispersed in 40 mL of distilled water and the pH was adjusted to 10.5, stirred at 150 rpm (1, 2, 3 h) at 30 °C, then centrifuged at 10,000 g, for 15 min. The insoluble residue was removed with a cloth filter, all the supernatants were combined, and the pH was adjusted for protein precipitation at the isoelectric point (pH 4.5) and left overnight. The RBPC was prepared as before and stored in PE bags at 5 °C.

The effect of different salt solutions (0, 0.1, or 0.5 M) was compared by extracting the RBPC for 1 h at 30 °C. The dispersion was initiated using 1:4 (w/v) DRB: salt solution (0.1 M, 0.5 M), homogenized (13,500 rpm, 1 min) using an open system homogenizer (Ultra-Turrax T25 basic, IKA-WERKE, Germany), then the pH was adjusted to the required pH (8, 9, 10). The slurry was shaken, centrifuged for 15 min, separated using a cloth filter. Then,

the pH was adjusted for protein precipitation at an isoelectric point and the protein solution was centrifuged (15 min), washed, neutralized, frozen overnight, and freeze-dried for 96 h before storage in zip-lock PE bags at 5 °C. Analysis of slurry prepared using 0.1 M solution at 40 °C and 50 °C was conducted for comparison.

2.4 Basic chemical composition of rice bran and rice bran protein concentrates (RBPC)

The approximate basic chemical composition was determined following the methods of the Association of Official Agricultural Chemists [12]. Specifically, protein content and yield were measured by the standard Kjeldahl's method [12] using a value of 5.95 as a protein conversion factor. The RBPC yield was calculated according to Wang et al. [6]:

$$\text{Yield (\%)} = \frac{\text{weight (g) of RBPC} \times \text{protein content (\%) of RBPC}}{\text{10 g (weight of DRB)} \times \text{protein content (\%) of DRB}} \times 100 \quad (1)$$

2.5 Statistical analysis

All the experiments were performed in triplicate and the means were calculated. Differences between groups were assessed by one-way ANOVA followed by Tukey's multiple range test using Minitab 18 software. All data were expressed as mean ± standard deviation and a $p \leq 0.05$ was considered significant.

3. Results and discussion

3.1 Proximate analysis

The proximate composition of full fat and DRB is presented in Table 1, showing significant differences in moisture content, ash, fat, and fiber after the defatting process. Gadalkar et al. [13] reported that rice bran constitutes 12–23% oil which can be removed by hexane without affecting the protein content. The fat content was similar to that reported by Jiamyangyuen et al. [2] and Gnanasambandam and Hettiarachchy [14]. The protein content was 9–20% [3] but reported as low-protein content [15].

Table 1 Proximate composition of full fat and defatted rice bran.

Material	Moisture (%)	Ash (%)	Crude Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)
Full Fat	7.49 ± 0.06^{B}	7.16 ± 0.18^{B}	11.66 ± 0.48^{A}	13.54 ± 1.15^{A}	9.18 ± 0.19^{A}	50.97 ± 1.83^{A}
Defat	9.85 ± 0.17^{A}	7.56 ± 0.07^{A}	13.42 ± 5.56^{A}	3.29 ± 0.49^{B}	8.66 ± 0.26^{B}	57.22 ± 5.83^{A}

Values superscripted with dissimilar letters A are significantly different ($p < 0.05$).

3.2 Enzymatic extraction

The protein content and yield of RBP extracted by xylanase were not significantly different from the control (Table 2). Xylan is a common plant cell wall polysaccharide contained in the RBP which is released when the cell wall is disrupted [6]. Xylanase hydrolyzes xylan from D-xylose (D-xylopyranosyl) to short-chain xylooligosaccharides. More intercellular constituents such as protein will be liberated by cleaving the linkages within the polysaccharide matrix. The increment of 4% protein content after xylanase treatment was lower than reported by Wang et al. [6] and could be attributed to the different compositions and varieties used in the extraction, such as the rice bran cultivar, type of enzyme, and the application of speed and centrifugation time. Unfortunately, the RBPC extract was low in protein and yield.

Table 2 Protein content and yield of enzymatic extraction.

Sample	Protein content (%)	Protein Yield (%)
Control	43.30 ± 2.79^{A}	19.16 ± 8.92^{A}
Xylanase	47.17 ± 0.98^{A}	19.80 ± 10.88^{A}

Values superscripted with dissimilar letters A are significantly different ($p < 0.05$).

3.3 Optimization of RBPC alkaline extraction

The protein content and yield of RBPC at different pH (8–10) and temperatures (30–50 °C) are shown in Table 3 and are in line with those reported by Prakash and Ramanatham [19]. Surprisingly, the protein yield was 8.9% higher compared to Jiamyangyuen et al. [2] and Yadav et al. [16], but lower compared to Gupta et al. [11] and Gnanasambandam and Hettiarachchy [14]. These differences could be explained by the different cultivars used in each study [5]. DRB of crude protein found in Gupta et al. [11] (17%) and Gnanasambandam and Hettiarachchy [14] (15.32%) were higher compared to the present study (Table 1).

Table 3 Percentage of protein content and yield at different pH (8-10) and temperature (30-50 °C).

Temperature (°C)	pH	Protein Content (%)	Protein Yield (%)
30	8	41.51 ± 0.59^{A}	10.34 ± 0.95^{A}
40	8	42.34 ± 0.98^{A}	8.76 ± 0.16^{A}
50	8	41.51 ± 1.77^{A}	10.29 ± 0.15^{A}
30	9	44.29 ± 0.20^{A}	21.80 ± 10.0^{A}
40	9	40.40 ± 2.95^{A}	13.23 ± 0.09^{A}
50	9	40.26 ± 5.10^{A}	11.15 ± 4.69^{A}
30	10	40.96 ± 1.37^{A}	16.82 ± 0.16^{A}
40	10	41.93 ± 0.00^{A}	19.97 ± 2.25^{A}
50	10	42.21 ± 0.00^{A}	21.19 ± 0.28^{A}

Values superscripted with dissimilar letters A are significantly different ($p< 0.05$).

The highest protein content was extracted at 30 °C, pH 9 in line with Gupta et al. [11]. The protein content at pH 8 and 9 slightly reduced as the temperature increased due to the extraction of non-protein components. Gupta et al. [11] also reported that an increase in temperature (30–75 °C) at pH 9.5 decreased the protein content and increased the protein yield. In contrast, the protein content and yield increased at pH 10 as temperature increased because of the improved protein solubility and swelling of the starch granules. Furthermore, Coa et al. [4] claimed that heat treatment (20-50 °C) might partially break down the hydrogen and disulfide bonds, improving the protein dissolution rate in germinated brown rice.

The protein yield increased with pH, with the highest protein yield (30–80%) obtained at pH 7–12 due to the glutenin readily dissolving in the alkaline extraction medium. The highly alkaline solutions disrupt the structure of the protein, starch, and phytate, hence increasing the protein yield [3,4,9]. As shown in Table 3, the highest protein content and yield were obtained at pH 9 and 30 °C, which is in line with previous studies [2,17,18]. Therefore, further analysis of the alkaline extraction was performed at fixed pH 9 and a constant temperature of 30 °C.

The effects of particle size, solvent ratio, centrifuge time, filter cloth, supernatant leftover night, and 72 h extraction on protein content and yield are shown in Table 4. The particle size had no significant effect on protein content in contrast to Gnanasambandam and Hettiarachchy [14] and Jiamyangyuen et al. [2] who reported that a smaller particle size yields a higher protein content. The 0.4 mm particles produced the highest protein yield in line with Prakash and Ramanatham [19], and Gnanasambandam and Hettiarachchy [14]. The use of fine particles has an important implication for increasing protein extractability. An increase in extracted protein extraction as the particle size of the starting material decreases indicates that smaller particles contribute to a more efficient protein extraction rate, therefore more significant total protein mass transfer during the initial alkaline solubilization [18,20]. Nevertheless, a very fine particle size (200 mesh) leads to contamination of the extract with non-protein components such as cellulose, hemicellulose, pentosans, and lignin [14].

There was no difference in the protein content but a significant difference in protein yield obtained at different solvent ratios (w/v) (Table 4), with the 1:4 solvent ratio (80 mesh) yielding the highest protein content and yield. The increase in protein yield was because of the higher concentration gradient due to the availability of fresh solvent enhancing the mass transfer rate [13]. Thus, a decrease in protein yield is attributed to the excess dilution of solute, thereby reducing the mass transfer gradient [21]. The 1:4 (w/v) solute-solvent ratio would reduce production costs and lower waste disposal for industrial-scale applications [22,23].

Table 4 Percentage of protein content and yield for factors: particle size, solvent ratio, centrifuge time, filter cloth, supernatant leftover night, 72 h extraction.

Factor	30 °C, pH 9	Protein Content (%)	Protein Yield (%)
Particle size	0.177mm (80 Mesh) 1:4	51.23 ± 0.59^{A}	26.85 ± 1.54^{A}
	0.4 mm, 1:4	45.87 ± 3.77^{A}	16.74 ± 1.84^{AB}
	0.177mm (80 Mesh), 1:4	51.23 ± 0.59^{A}	26.85 ± 1.54^{A}
Solvent ratio	0.4 mm, 1:4	45.87 ± 3.77^{A}	16.74 ± 1.84^{AB}
	0.4 mm, 1:6	50.26 ± 0.00^{A}	13.30 ± 0.06^{B}
	0.4 mm, 1:10	42.90 ± 5.69^{A}	15.95 ± 12.07^{AB}
Centrifugal time, cloth filter (CF) and supernatant without left over night (WOO)	0.4 mm, CF, WOO, 15 min,	51.37 ± 0.00^{A}	13.30 ± 0.60^{B}
	0.4 mm, CF, WOO, 30 min	43.32 ± 6.28^{A}	17.18 ± 1.81^{AB}
Extraction 72 h	0.4 mm, CF, WOO, 25 min	5.62 ± 0.53^{B}	1.84 ± 0.55^{C}

Ratio refers to solute: solvent (w/v); Values superscripted with dissimilar letters A are significantly different ($p< 0.05$).

The 15 min centrifugation yielded the highest protein content (51.37%) but the lowest yield (13.30%) (Table 4). The lower protein content and yield observed after the 72 h extraction might be due to dissoluble protein as the pH dropped near the isoelectric point. Mechanical shear generated by vigorous shaking for a long time or when the pH shifts to a very low value (between pH 2–5) increases the protein net charge and the strong intramolecular electrostatic repulsion causes swelling and unfolding of the protein molecules, i.e., protein denaturation [24].

A comparison of the single versus double extraction is provided in Table 5. The protein content was slightly reduced with increased stirring time for the single extraction but increased for the double extraction, with the highest protein content observed at 1 h stirring for the single extraction and 3 h stirring for the double extraction. Since the highest protein content was obtained from 1 h stirring (single extraction), the extraction conditions for all further analyses were a fixed solvent ratio of 1:4, 15 min centrifugal time, and constant temperature of 30 °C at pH 9.

Table 5 Percentage of protein content and yield of different extraction of stirring time for single and re-extraction of DRB.

Factor	Protein Content (%)	Protein Yield (%)
Single extract, 1h stir, WOO	43.32 ± 6.28^{A}	16.95 ± 0.20^{A}
Single extract, 2h stir, WOO	41.93 ± 9.42^{A}	16.93 ± 1.44^{A}
Single extract, 3h stir, WOO	37.76 ± 0.79^{A}	17.52 ± 2.13^{A}
Re-extract, 1h stir, overnight	26.10 ± 6.28^{A}	29.83 ± 6.77^{A}
Re-extract, 2h stir, overnight	27.50 ± 1.18^{A}	31.46 ± 13.30^{A}
Re-extract, 3h stir, overnight	39.57 ± 0.98^{A}	30.24 ± 1.08^{A}

Values superscripted with dissimilar letters A are significantly different ($p < 0.05$).

The effects of different salt solutions on the protein content and yield are provided in Table 6-7. Chen and Houston [25] found an increment in the percent protein content with increased pH. The highest percent protein content was obtained at both homogenized and pH 10, 0.5 M NaCl, 30 °C, 49.84±3.73%, followed by 47.48 ± 5.10% for 0.1 M NaCl, 50 °C due to the increased solubilization of protein and total solid [3,4], [9,25]. The average percent protein content of both homogenized 0.5 M NaCl, 30 °C and 0.1 M, 50 °C were similar at pH 8 and 9 but lower than the control, pH 9, 30 ºC. Accordingly, at constant pH and ionic strength, the protein solubility will increase with temperature between 0 °C and 40–50 °C [24,26]. A lower protein content was obtained for extraction at homogenized 40 °C, 0.1 M salt at all pH compared to 50 °C, 0.1M salt. Similar to Ansharullah et al. [27], a higher protein content was obtained at 50 °C (53.20%) than 40 °C (43.07%) at neutral pH. Gupta et al. [11] found that protein extracted at pH 9 in different alkaline extraction temperatures (°C 30, 45, 60, 75) showed low to high solubility than protein extracted at 75 °C < 45 °C < 60 °C < 30 °C at all pH (pH 7, 8, 9, 10) with significant percentage protein 71, 76.7, 78.0 and 79.9% respectively. Similar to Bandyoadhyay et al. [28] at pH 10, 50–55 ºC, the protein content was higher at 86.2%.

Table 6 Protein content of extraction from homogenized RBPC with salt solution (0.1 M, 0.5 M).

pH	Protein Content (%)				
	Control, 30 °C	0.1 M, 30 °C	0.5 M, 30 °C	0.1 M, 40 °C	0.1 M, 50 °C
8	43.17 ± 0.59^{A}	39.98 ± 2.75^{A}	35.96 ± 2.16^{A}	33.04 ± 6.68^{A}	43.87 ± 9.42^{A}
9	45.26 ± 0.39^{A}	43.32 ± 4.32^{A}	44.43 ± 3.14^{A}	35.96 ± 2.16^{A}	44.15 ± 9.82^{A}
10	45.81 ± 12.17^{A}	43.59 ± 3.53^{A}	49.84 ± 3.73^{A}	38.60 ± 4.32^{A}	47.48 ± 5.10^{A}

Values superscripted with dissimilar letters A are significantly different ($p < 0.05$). Control – DRB extracted under of alkaline condition, no pre-treatment of homogenizing, 0 M NaCl, at temperature 30 °C.

Table 7 Protein yield of extraction from homogenized RBPC with salt solution (0.1 M, 0.5 M).

pH	Protein Content (%)				
	Control, 30 °C	0.1 M, 30 °C	0.5 M, 30 °C	0.1 M, 40 °C	0.1 M, 50 °C
8	25.00 ± 0.02^{BC}	12.76 ± 3.79^{C}	15.80 ± 2.13^{BC}	15.02 ± 5.78^{C}	12.24 ± 2.40^{C}
9	28.90 ± 1.34^{AB}	13.33 ± 0.51^{BC}	20.91 ± 7.88^{BC}	12.59 ± 2.50^{C}	19.58 ± 6.13^{BC}
10	41.52 ± 0.40^{A}	22.58 ± 0.84^{BC}	24.24 ± 0.97^{BC}	20.87 ± 0.83^{BC}	24.40 ± 0.35^{BC}

Values superscripted with dissimilar letters A are significantly different ($p < 0.05$). Control – DRB extracted under of alkaline condition, no pre-treatment homogenizing, 0 M NaCl, at temperature 30 °C.

Generally, the Osborne solubility fraction of rice bran protein typically provides a significant amount of glutelin proteins that are usually solubilized with a high alkaline concentration. Despite the large amounts of glutelin contained in rice bran (22%) [18], the alkali must break the hydrogen, amide, and disulfide bonds for ready extraction in alkaline conditions, reducing its molecular size and aggregation to render it soluble. The substantial reduction in the size of glutelin molecules during alkaline solubility results in its extraction along with albumin and globulin [7,9,13], [25,29,30]. Moreover, the solubility of glutelin increases due to the dissociation aggregates of proteins in alkaline solutions.

However, at 30 °C, the protein content in the control was slightly reduced for both pH 9 and 10 at 0.1 M salt and slightly increased at 0.5 M. The increasing salt could explain this, reducing the solubility of glutelin with low solubility or insoluble in NaCl solution compared to albumin and globulin, which are expected to be easily soluble.

Besides, glutelin was efficiency extracted at pH higher than 10 [3,13,22,30-32]. Furthermore, alkaline at pH 8, near the isoelectric point, might not provide sufficient alkali needed to promote protein-solvent interactions.

Moreover, albumin and globulin are extractable under salinity-alkaline assistance, which gives the effect of salting in and protein aggregate dissociation in alkaline conditions increases the overall solubility of albumins and

globulins resulting in more extracted proteins compared to near neutral pH conditions. The salinity of 0.5–1.0 M NaCl may increase the solubility of proteins, promoting protein-solvent interactions through chloride ions, thereby increasing the solubility by electrostatic repulsion after binding to the positively charged protein groups and hydration of charged residues, which promotes protein solubilization into the extraction solvent [12,26,33]. This explains the increase in percentage protein content with 0.5 M salt at 30 °C.

However, there was a significant difference in RBPC yield between the control and homogenized 0.1 M (30–50 °C) and 0.5 M (30 °C) salt solutions. The homogenized extract and the use of saline solutions were insufficient to increase the yield as claimed by Phongthai et al. [5], Fabian and Ju [3], and Anderson and Guraya [34]. The shear force used for homogenization at 13,500 rpm, 1 min was unable to disrupt the cells to release proteins or enhance extraction under this condition as attained by Sun et al. [35] (1:15 (w/v), pH 9.5, 40 °C) who homogenized at 10,000 rpm for 5 min increasing the yield to 52.83% compared to the 49% control. Furthermore, Anderson and Guraya [34] used colloid milling from DRB followed by homogenization to increase the protein extracted from 13.9% to 14.7%, with a further increase to 16.5% after homogenization for 10 minutes using pressure ~1.7×104 kPa.

The highest RBPC yield (41.52±0.40%) was obtained under the control conditions (30 °C, pH 10). The protein yield at pH 9 was significantly higher than that observed in Table 4 (16.74%) and Table 5 (17%), which were under the same extraction condition (30 °C, pH 9,1:4 (w/v), 10,000 g, 20 min centrifugation). This variation might be due to the different particle size distribution with the different meshes. The use of salt did not significantly affect the yield as claimed by Paraman et al. [22], even when the extract was homogenized before exposure to the salt solution. The control (30 °C) conditions at all pH resulted in higher protein extractions compared to the treated extracts within the same pH. Glutelin, which was soluble under this condition, could explain the high yield, especially at pH 10 close to the actual pH extractability. At 30 °C, the protein yield was higher at 0.5 M than 0.1 M salt at every pH similar to Gadalka et al. [13]. The variation in temperatures at 0.1 M salt concentration was similar to Paraman et al. [22], that is, the protein yield increased with temperature, with no significant difference between 30 °C and 50 °C.

4. Conclusion

RBPC extraction via enzymatic and alkaline extraction methods is feasible, extracting a minimum of 43.3% protein content. The optimal alkaline extraction conditions for the extraction of rice bran protein (protein content 43–51%, yield 13–29%) were found to be pH 9 at 30 °C using 0.4 mm particles in a 1:4 solvent ratio, then centrifugation for 15 mins and use of a cloth filter.

5. Acknowledgements

The research work was funded by the Putra Grant UPM (Grant No. 9550600).

6. References

[1] Al-Doury MKW, Hettiarachchy NS, Horax R. Rice-endosperm and rice-bran proteins: a review. J Am Oil Chem Soc. 2018;95(8):943-956.
[2] Jiamyangyuen S, Srijesdaruk V. Extraction of rice bran protein concentrate and its application in bread. Songklanakarin J Sci Technol. 2005;27(1):55-64.
[3] Fabian C, Ju YH. A review on rice bran protein: its properties and extraction methods. Crit Rev Food Sci Nutr. 2011;51(9):816-827.
[4] Cao X, Li C, Wen H, Gu Z. Extraction technique and characteristics of soluble protein in germinated brown rice. Int J Food Prop. 2010;13(4):810-820.
[5] Phongthai S, Homthawornchoo W, Rawdkuen S. Preparation, properties and application of rice bran protein: a review. Int Food Res J. 2017;24(1):25-34.
[6] Wang M, Hettiarachchy NS, Qi M, Burks W, Siebenmorgen T. Preparation and functional properties of rice bran protein isolate. J Agric Food Chem. 1999;47(2):411-416.
[7] Adebiyi AP, Adebiyi AO, Hasegawa Y, Ogawa T, Muramoto K. Isolation and characterization of protein fractions from deoiled rice bran. Eur Food Res Technol. 2009;228(3):391-401.
[8] Momen S, Alavi F, Aider M. Alkali-mediated treatments for extraction and functional modification of proteins: critical and application review. Trends Food Sci Technol. 2021;11:778-797.
[9] Hamada JS. Characterization of protein fractions of rice bran to devise effective methods of protein solubilization. Cereal Chem. 1997;74(5):662-668.
[10] Shen L, Wang X, Wang Z, Wu Y, Chen J. Studies on tea protein extraction using alkaline and enzyme methods. Food Chem. 2008;107(2):929-938.
[11] Gupta S, Chandi GK, Sogi DS. Effect of extraction temperature on functional properties of rice bran protein concentrates. Int J Food Eng. 2008;4(2):1165.
[12] Association of Official Analytical Chemist. Official Methods of Analysis. 15th ed. Washington: USA; 1990.

[13] Gadalkar SM, Gogate PR, Rathod VK. Recovery of proteins from rice mill industry waste (rice bran) using alkaline or NaCl-assisted alkaline extraction processes. J Food Process Eng. 2017;40(3):1-13.
[14] Gnanasambandam R, Heitiarachchy NS. Protein concentrates from unstabilized and stabilized rice bran: preparation and properties. J Food Sci. 1995;60:1066-1069.
[15] Prakash J. Rice bran proteins: properties and food uses. Crit Rev Food Sci Nutr. 1996;36(6):537-552.
[16] Yadav RB, Yadav BS, Chaudhary D. Extraction, characterization and utilization of rice bran protein concentrate for biscuit making. Br Food J. 2011;113(9):1173-1182.
[17] Theerakulkait C, Chaiseri S, Mongkolkanchanasiri S. Extraction and some functional properties of protein extract from rice bran. Kasetsart J Nat Sci. 2006;40:209-214.
[18] Betschart A, Fong RY, Saunders R. Rice by-products: comparative extraction and precipitation of nitrogen from U.S. and Spanish bran and germ. J Food Sci. 1977;42(4):1088-1093.
[19] Prakash J, Ramanatham G. Effect of stabilization of rice bran on the extractability and recovery of proteins. Mol Nutr Food Res. 1994;38(1):87-95.
[20] Russin TA, Arcand Y, Boye JI. Particle size effect on soy protein isolate extraction. J Food Process Preserv. 2007;31(3):308-319.
[21] Akter D, Begum R, Rahman MN, Talukder N, Alam M J. Optimization of extraction process parameter for rice bran protein concentrate and its utilization in high protein biscuit formulation. CRNFSJ. 2020;8(2):596-608.
[22] Paraman I, Hettiarachchy NS, Schaefer C. Preparation of rice endosperm protein isolate by alkali extraction. Cereal Chem. 2008;85(1):76-81.
[23] Cheetangdee N. Effects of rice bran protein hydrolysates on the physicochemical stability of oil-in-water emulsions. J Oleo Sci. 2014;63(12):1231-1241.
[24] Damodaran S. Amino acids, peptides, and proteins. In: Damodaran S, Parkin KL, editors. Fennema's Food Chemistry. 5th ed. Florida: Taylor & Francis Group; 2017. p. 269-295.
[25] Chen L, Houston DF. Solubilization and recovery of protein from defatted rice bran. Cereal Chem. 1970;4:72-79.
[26] Zayas JF, editor. Solubility of Proteins. In: Zayas JF, editor. Functionality of proteins in food. 1st ed. Heidelberg: Springer; 1997. p 6-75.
[27] Ansharullah A, Hourigan JA, Chesterman CF. Application of carbohydrases in extracting protein from rice bran. J Sci Food Agric. 1997;74(2):141-146.
[28] Bandyopadhyay K, Misra G, Ghosh S. Preparation and characterization of protein hydrolysates from Indian defatted rice bran meal. J Oleo Sci. 2008;57(1):45-52.
[29] Horax R, Hettiarachchy N, Kannan A, Chen P. Protein extraction optimisation, characterisation, and functionalities of protein isolate from bitter melon (Momordica charantia) seed. Food Chem. 2011;124(2):545-550.
[30] Adebiyi AP, Adebiyi AO, Ogawa T, Muramoto K. Preparation and characterization of high-quality rice bran proteins. J Sci Food Agric. 2007;87(7):1219-1227.
[31] Delcour JA, Hoseney RC. Review of principle of cereal science and technology. J Agric Food Inf. 2010;11(3):256-257.
[32] Tecson EMS, Esmama BV, Lontok LP, Juliano B. Studies on the extraction and composition of rice endosperm glutelin and prolamin. Cereal Chem. 1971;48(2):168-181.
[33] Lestari D, Mulder W, Sanders J. Improving Jatropha curcas seed protein recovery by using counter current multistage extraction. Biochem Eng J. (2010);50(1-2):16-23.
[34] Anderson AK, Guraya HS. Extractability of protein in physically processed rice bran. J Am Oil Che Soc. 2001;78(9):969-972.
[35] Sun LH, Lv SW, He LY. Comparison of different physical technique-assisted alkali methods for the extraction of rice bran protein and its characterizations. Int J Food Eng. 2017;13(10):1-12.