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ORIGINAL PAPER

Effect of different degrees of milling on the protein composition in brown rice brans

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Abstract

In this study, the rice protein (RP) fractions (albumin, globulin, glutelin, and prolamin) in rice bran were obtained through the sequential extraction method using 2% (w/w) NaCl, 0.1 N NaOH, and 70% (w/w) ethanol and the proportions of water-soluble protein fractions (albumin) were compared among different degrees of milling (DM; 3%, 5%, 7%). The three types of bran showed different chemical components and populations of the four RP fractions. The 7% DM of brown rice is the stage at which the bran layers are completely removed and white rice is produced. In the 7%-DM bran, the content of water-soluble albumin was the highest**.** The extracted RP fractions contained eight essential amino acids and among them, albumin had the highest content of lysine. The secondary structures measured by circular dichroism spectroscopy were rich in alpha-helices. These results provide basic data for the extraction and utilization of water-soluble proteins from rice bran.

Keywords Rice bran · Rice protein · Water soluble protein · Degree of milling · Albumin

Introduction

The need to develop new sources of plant proteins to meet global demands in economical and sustainable ways has increased with global population growth. A growing number of consumers eat mostly vegetables for health reasons, and efforts are being made to replace animal protein with plant protein to minimize risk of bovine spongiform encephalitis and to reduce environmental impacts of livestock farming.

Although soybean and wheat remain primary plant protein sources, rice has emerged as an alternative source owing to consumer aversion to genetically modified organisms and increasing prevalence of gluten allergies. According to a review by Tran et al. [1], rice, soybean, and pea proteins dominate the supplement market, but soybean and pea protein allergens make them less desirable for consumption. Rice protein (RP) is nutritious and hypoallergenic, and exhibits various beneficial physiological activities, including cholesterol lowering, antioxidative, and anticancer effects. It is thus considered a healthy protein source and, importantly, is recognized as suitable for processed foods intended for infants and young children, whose immune systems are not fully established [2, 3].

Brown rice is obtained by simply dehusking the harvested rice paddy. To obtain white rice (rice endosperm), the bran and embryo are removed from brown rice through a process called milling, where the extent of bran removal is termed degree of milling (DM). Rice bran, a fine powdery by-product ground from the surface of brown rice, consists of pericarp, seed coat, nucellus, aleurone, sub-aleurone, some starchy endosperm, enzymes, and any remaining hull. Ninety percent of the rice bran obtained during milling is used as livestock feed. Rice bran oil is extracted from the remainder.

Rice bran contains approximately 11% minerals, including iron, phosphorus, and magnesium, 13% crude protein, 20% dietary fiber, and 20% oil. Rice bran composition can vary with several factors, including rice variety, climate conditions, and rice processing methods. Rice bran is considered a rich source of natural antioxidants and bioactive compounds, including essential amino acids, phenols, vitamin E,

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and γ-oryzanol, which have been reported to confer diverse potential human health benefits. For these reasons, there is a growing interest in using rice bran in the food industry [3–5].

Generally, RPs can be divided into two categories: rice bran protein (RBP) and rice endosperm protein (REP), which differ in composition and proportions of four protein fractions: water-soluble albumin, salt-soluble globulin, alkali/acid-soluble glutelin, and alcohol-soluble prolamin [6]. REP consists mostly of glutelin (accounting for ≥ 80% of total protein), with approximately 5–10% prolamin and some albumin and globulin. In contrast, RBP has relatively high water-soluble albumin and globulin content, with previous studies reporting average compositions of 34% albumin, 15–26% globulin, 4% prolamin, and 11–27% glutelin [7, 8].

Although RP is recognized as having good qualities, its application in the food industry has been limited due to its poor water solubility, which reduces its functionality. Given that rice bran contains approximately 60% water-soluble protein fractions such as albumin and globulin, it is logical to find ways to utilize RBP as a protein source. Because rice bran is usually discarded as a by-product, its practical use would also be beneficial in terms of resource recycling.

The nutritional characteristics and applications of RBP are predicted to differ from those of REP due to inherent differences in composition. Considering that RBP has a higher water-soluble protein content, it is expected to have better functionality than REP in emulsification, gelation, and foaming capacities [9].

The nature and composition of rice bran differ depending on rice cultivar, milling method, and DM. DM affects composition due to different distribution of the four protein fractions within the rice bran layers. According to previous studies, different concentrations of compounds can be obtained from the different bran layers [10, 11].

Therefore, details regarding what proportions of the various protein fractions in rice bran can be obtained with different DMs, and which layer of rice bran is richest in water-soluble proteins would be useful for practical extraction of useful proteins from rice bran. The aim of this study was to determine which parts of rice bran are retained with different DMs, and how the compositions of the different brans obtained differ. In addition, the RBPs of each bran were characterized.

Materials and methods

Materials

Sinseonchal brown rice (a waxy variety) was purchased from Yuga Noghyup (Daegusi, Republic of Korea). The dietary fiber assay kit and dialysis tubing (molecular weight cut-off, 10 kDa) were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Laboratory milling

Brown rice milling was performed using a laboratory milling machine (MC-90A, Toyo Rice Cleaning Machine Co. Ltd., Wakayama, Japan). The DM was adjusted to 3%, 5%, and 7% by varying the duration of milling and checking the mass of the rice before and after milling, where the percentage loss in mass corresponded to the DM. The 3%-, 5%-, and 7%-DM brans were stabilized by dry heat treatment. 7% DM was selected to represent the bran obtained after white rice was produced; at 3% and 5% DM, 50% and 80% of brown rice were removed, respectively. To determine how the four rice protein fractions were distributed within the bran, the experiments were carried out at 3%, 5%, and 7% DM.

Stabilization of the rice brans

During the brown rice milling process, the bran and embryos were separated with a 40-mesh vibrator. The separated rice bran was heated at 120 °C for 10 min to inactivate lipases and lipoxygenases and then cooled. The heating step was carried out in a confined space with dry heat to minimize damage to the proteins, dietary fiber, starch, and inorganic matter in the bran.

Proximate analysis

The crude protein and lipid contents were determined using the Kjeldahl and the Soxhlet extraction method, respectively (AACCI, 2012) [12]. To calculate protein contents, a nitrogen coefficient of 6.25 was used for all samples. The total starch content was determined using a total starch kit (K-TSTA Megazyme International Ltd., Wicklow, Ireland) [13], and the total dietary fiber content was determined using AOAC Method 985.29 (1997) [14].

To analyze the total dietary fiber content, the sample (1.0 g, d.b.) was dispersed in 40 mL of phosphate buffer (pH 6.0) in a tall form beaker and heat stable α-amylase (100 μL) was added. The beaker was placed in a boiling water bath for 20 min with continuous stirring. After cooling to room temperature, the reactant was adjusted to pH 7.5 ± 0.2 with 0.275 N NaOH in a 60 °C water bath and protease (100 μL) was added and incubated for 30 min with agitation. The reactant was adjusted to pH 4.0–4.6 with 0.325 M HCl and amyloglucosidase (100 μL) was added. After incubating for 30 min, 4 volumes of 95% ethanol were added and the mixture was allowed to stand for 1 h at room temperature. The precipitate was collected on a bed of celite onto a glass crucible (porosity no. 2) with constant weight. The insoluble

residue was washed with 78% ethanol, 95% ethanol, and acetone. The crucible with the residue was dried in a 105 °C oven and weighed, and total dietary fiber content was calculated.

Fractionation of rice bran protein

The four constituents of RBP were extracted using the Method 3 reported in a previous study [7], with slight modification. In brief, 20 g of each stabilized rice bran sample was reacted sequentially in the following solutions: 120 mL of 2% (w/w) NaCl; dialysis against distilled water; 0.1 N NaOH; and 70% (w/w) ethanol. For each extraction solution, the samples were stirred for 1 h and then centrifuged at 2569×*g* for 15 min. The supernatants were collected and the precipitants were extracted one more time. The first and second supernatants were freeze-dried after collection. Prolamin was precipitated by the addition of acetone, and the precipitated glutelin was adjusted to pH 7. The yield of the four protein fractions was obtained by measuring the mass of the freeze-dried isolates, and the percentage ratio was the proportion of each protein fraction relative to the total protein yield. For comparison, milled rice flour protein was isolated from waxy rice using 0.2% NaOH [15].

Amino acid composition

The various rice bran samples were first subjected to HCl hydrolysis, following which their amino acid compositions were measured using an automatic amino acid analyzer (S433, Sykam GmbH, Eresing, Germany) equipped with a cation separation column (4.6 × 150 mm, Sykam LCA K 06/Na). The column temperature was set to 57–74 °C, the flow rates of the buffer and reagent were 0.45 and 0.25 mL/min, respectively, the buffer pH range was 3.45–10.85, and the fluorescence spectrophotometer was set to record at 440 and 570 nm wavelengths.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis

The four protein fractions extracted from the various rice brans were characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Each fraction was first solubilized in citrate–phosphate buffer (pH 12) and then centrifuged at 925×*g* for 10 min to remove any insoluble material. An aliquot of the supernatant was mixed with Laemmli sample buffer (Bio-Rad Laboratories Inc., Hercules, CA, USA) and the mixture was heated for 5 min in a dry block heater, following which 15 µL was applied to the polyacrylamide gel (Mini-protean TGX precast gels, Bio-Rad Laboratories) for electrophoresis at 180 mA. The running buffer contained 25 mM Tris–HCl base (w/v), 192 mM glycine (w/v), and 0.1% (w/v) SDS. After electrophoresis, the gel was stained with 0.1% Brilliant Blue G in methanol/glacial acetic acid/water (4.5:1:4.5, v/v/v) and then destained with methanol/glacial acetic acid/water (2:1:7, v/v/v). The Prosi Prestained Protein Marker (Broad-range P8500, GenDEPOT, Katy, TX, USA), with a size range of 6–170 kDa, was used as the molecular weight marker.

Circular dichroism spectroscopy

The secondary structures of the protein fractions were analyzed using a circular dichroism (CD) spectrometer (Chirascan Plus, Applied Photophysics Ltd., Surrey, UK). The conditions used were as follows: ambient temperature, 190–250 nm detection range, 100 nm/min scanning speed, 0.1 nm sample cell optical path length, 100 mdeg/cm sensitivity.

Statistical analysis

All data presented are the mean ± standard deviation. Statistical analysis was performed using the SPSS software package (version 21.0, SPSS Inc., Cary, NC, USA). Tukey's honestly significant difference test was used to identify the statistical significance of differences between the samples ($p < 0.05$).

Results and discussion

Chemical composition of rice brans obtained with different degrees of milling

The chemical components of the rice brans generated with 3%, 5%, and 7% DM are shown in Table 1. The total

Table 1 Chemical composition of rice brans prepared with different degrees of milling

Bran type	Total starch	Protein	Fat	Total dietary fiber
3%-DM	0.82 ± 0.25[a]	15.26 ± 0.06[b]	17.63 ± 0.55[a]	46.80 ± 0.70[b]
5%-DM	0.92 ± 0.11[a]	15.10 ± 0.09[b]	20.10 ± 0.90[b]	46.54 ± 1.22[b]
7%-DM	1.87 ± 0.36[b]	14.82 ± 0.06[a]	20.87 ± 0.65[b]	41.61 ± 1.14[a]

[a,b]Values in the same column marked with a different letter are significantly ($p < 0.05$) different by Tukey's honestly significant different test

starch, protein, fat, and total dietary fiber contents were in the range of 0.82–1.87%, 14.82–15.26%, 17.63–20.87%, and 41.61–46.80%, respectively, consistent with the values reported by other researchers (i.e., 34–54% carbohydrates, 13–17% protein, 13–23% fats, and 6–14% fiber) [16–18]. The protein and total dietary fiber contents decreased with increasing DM, whereas the total starch and fat contents increased. Specifically, the 7%-DM bran had lower protein and slightly higher total starch amounts than the 3%- and 5%-DM brans.

Because the bran and embryo together account for approximately 8% of the total weight of brown rice, and the bran alone accounts for approximately 6% of that weight, a 7% DM means that all the bran layers are removed and only white rice is left. Thus, the slight starch increase in the 7%-DM bran might have been due to inflow from the breakage of the endosperm. Other studies have suggested that the decreasing trend in bran protein content with increasing DM might be attributed to an influx of starch from the endosperm [1, 10, 19]. Essentially, the proteins of rice bran are sequestered within the cell in the form of protein bodies (PBs) of which there are two morphologically distinct types: spherical and crystalline. The spherical PBs are enriched in prolamin, whereas the crystalline ones contain mainly glutelin and globulin [9, 20]. The proteins of brown rice are concentrated in the aleurone layer, the embryo, and the sub-aleurone layer of the endosperm, and the two types of PBs are deposited mainly in the sub-aleurone layer where starch granules are deposited prior to the large spherical PBs in the developing endosperm [9, 20, 21].

Previous studies have reported protein contents of 11.7–15.2% in the aleurone and sub-aleurone layers and 0.5–8% in the pericarp layer, seed coat, and nucleus layer. Based on these data, the 3%- and 5%-DM brans in the present study contained the aleurone layer, whereas the 7%-DM bran with its lower protein content likely included a slight amount of starchy endosperm and the sub-aleurone layer that surrounds it.

Tran et al. [22] reported that the lipid content of the by-product of the first and second rice milling processes was nearly three times higher (at ~ 22%) than that from the third milling process (~ 8%), which were consistent with the reported lipid contents of 21–39% in the rice embryo and 16.6% in the rice aleurone layers. In our study, despite that the embryo by-product had been removed during milling by sieving, the fat content was still relatively high at 17.6–20.9%.

Effect of the degree of milling on the yield of each protein fraction

The yields of the various protein fractions in the 3%-, 5%-, and 7%-DM rice brans are shown in Table 2. The total protein yields were in the range of 9–10%. Considering that the protein content of bran is in the range of 10–16%, it would appear that most of the proteins had been extracted.

Although there was no significant difference between 5% DM and 7% DM, the yield of albumin tended to increase as DM increased. By contrast, the yields of globulin, glutelin, and prolamin were not significantly different among the various brans ($p < 0.05$). Using a same sequential extraction method, Adebiyi et al. [7] obtained 27% albumin, 30% globulin, 42% glutelin, and 2% prolamin from rice bran, which were similar to the proportions in the 5%-DM bran of our study.

The amount of water-soluble protein fractions (i.e., albumin and globulin) increased from 43 to 65.4% with increasing DM. In the 7%-DM bran, the percentages of albumin, globulin, glutelin, and prolamin were 36.8%, 28.6%, 34.1%, and 0.5%, respectively. As described above, after 7% DM, the bran is almost completely removed and white rice is obtained. Therefore, it is expected that the bran obtained from this stage of milling would contain layers closely attached to the endosperm, including the sub-aleurone layer. Thus, the fact that the highest amount of water-soluble fractions occurred in the bran prepared with 7% DM suggests that the water-soluble protein content had increased because the bran layer was closer to the endosperm, including the sub-aleurone layer, which is assumed to be absent in the 3%- and 5%-DM brans. In other words, the differences in the proportion of each protein fraction in the three types of rice bran may be due to their distribution in the bran and

Table 2 Yield (g/20 g bran) and ratio (%) of protein fractions isolated from rice brans prepared with different degrees of milling

	3%-DM		5%-DM		7%-DM	
	Yield	Ratio	Yield	Ratio	Yield	Ratio
Albumin	0.33 ± 0.03^{a}	18.4	0.61 ± 0.04^{b}	29.8	0.68 ± 0.01^{b}	36.8
Globulin	0.44 ± 0.05^{a}	24.6	0.56 ± 0.00^{a}	27.3	0.53 ± 0.11^{a}	28.6
Glutelin	1.01 ± 0.25^{a}	56.4	0.86 ± 0.24^{a}	42.0	0.63 ± 0.11^{a}	34.1
Prolamin	0.01 ± 0.00^{a}	0.6	0.02 ± 0.01^{a}	1.0	0.01 ± 0.00^{a}	0.5
Total yield	1.79 ± 0.33	(9%)	2.05 ± 0.28	(10.1%)	1.85 ± 0.21	(9.3%)

[a,b]Values in the same row marked with a different letter are significantly ($p < 0.05$) different by Tukey's honestly significant different test

endosperm [22]. Schramm et al. [11] also obtained different concentrations of high-value compounds across the various bran layers, even though the extraction of pure bran fractions was difficult.

Prolamin proved difficult to isolate in the present study and its proportion in the three bran samples was very small. In fact, the rice bran content of prolamin has been reported to be only approximately 4% [9]. This protein fraction is soluble in 60–70% ethanol and readily soluble in acidic or alkaline solutions. The small proportion of prolamin isolated in the present study might be due to its co-extraction with the alkali-soluble protein glutelin. According to a previous study, prolamin and glutelin are distributed in the endosperm, whereas most of the albumin and globulin molecules are concentrated in the embryo and outer layers [23], which are possible reasons for the results obtained in our study. Another study reported that the by-products from the first and second milling processes have a low concentration of prolamin, whereas that from the third pass, which is rich in starchy endosperm, has relatively more prolamin [22]. However, in the present study, the amount of prolamin extracted from 7%-DM bran was still less than 1%.

Considering that the water insolubility of most of the RP fractions isolated from milled rice limits the range of their application in industry, rice bran, which is closely attached to the endosperm and has a relatively higher water-soluble protein content, can be an important source of soluble RPs. We surmise that new value-added opportunities can be created for rice bran, an abundant by-product of rice milling.

In summary, given that the DM of brown rice affects the ratios of the protein fractions of bran, it is an important factor that can be manipulated to obtain the desired composition of the different fractions for various purposes. In this study, approximately 65% of extractable protein in 7%-DM bran was composed of albumin and globulins, indicating that 65% of the protein in rice bran can potentially be extracted with water. Although globulins are salt-soluble proteins, a dilute globulin fraction can be extracted during water extraction for albumin. Moreover, the minerals in rice bran will also be dissolved during water extraction.

Characterization of the isolated rice protein fractions

SDS-PAGE patterns

The SDS-PAGE gel patterns of the RP fractions extracted from the three different rice brans are presented in Fig. 1. The protein fractions were composed of many subunits with different molecular weights. However, there was no difference in the gel patterns among the 3%-, 5%-, and 7%-DM bran samples, indicating that each protein fraction extracted from the three kinds of bran had the same molecular weight.

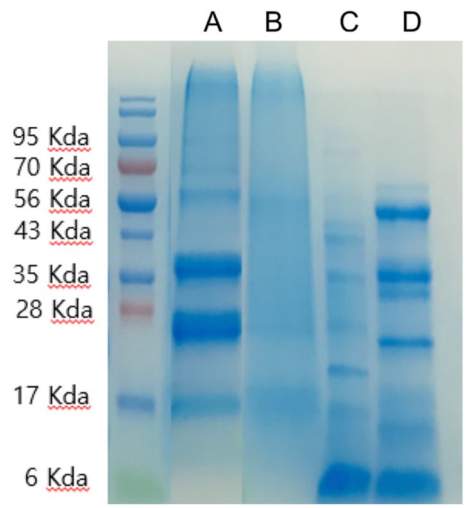

Fig. 1 SDS-PAGE gels of protein fractions extracted from 7%-DM bran and white rice. **A** white rice glutelin; **B** 7%-DM glutelin; **C** 7%-DM albumin; **D** 7%-DM globulin. *DM* degrees of milling

Tran et al. [22] also reported that each protein fraction of the three by-products of their rice milling passes had similar gel patterns. Furthermore, they suggested that the protein fractions had come from the same tissue sources and that each by-product of the three rice milling passes was a mixture of the same rice grain components.

Rice glutelin has been shown to be composed of two major polypeptide subunits of 30–40 kDa (α-subunit, acidic) and 19–23 kDa (β-subunit, basic) in size. The protein is synthesized as an approximately 51–57 kDa polypeptide precursor and is then hydrolyzed by enzymes to yield the α and β subunits. As shown in Fig. 1, the rice bran glutelin consisted of 17–20 kDa (β-glutelin), 28 kDa (α-glutelin), and 56 kDa (glutelin precursor) subunit bands on the SDS-PAGE gel, similar to the results in other studies, and glutelin extracted from white rice also had the same gel pattern [24, 25].

In this study, the main polypeptide subunits of albumin were 10, 17, 23, 28, 35, and 43 kDa in size, and minor subunits of greater than 43 kDa were also evident. Previous studies have demonstrated that rice albumin is a highly heterogeneous protein, with subunits ranging from 10 to 200 kDa, including predominantly 16 proteins and 60 glycoproteins [9, 16, 17, 26]. Agboola et al. [27] reported that rice albumin had six bands with molecular masses ranging from 15 to 56 kDa, which was consistent with our results. In the case of globulin, the main polypeptide subunits observed were approximately 10, 14–17, 25, 35, 40, and 55 kDa, and two minor subunits of approximately 17 kDa were also present. With regard to prolamin, other studies have reported its subunits to be small in size, such as 10, 13, and 16 kDa. However, no distinct prolamin band could be detected in our study. Similar to the results of this study, Wang et al. [28]

reported subunit profiles of > 35, 32, 31, 22, 17, and 14 kDa for albumin, 63, 53, 49, 36, and 21 kDa for globulin, and 60, 35, 22, and 13 kDa for glutelin. Any discrepancy in SDS-PAGE results among the various studies was likely due to differences in the extraction methods, rice species, and rice milling fractions used [28].

Amino acid composition

The amino acid composition of each protein fraction extracted from the various rice brans was examined and compared with those of proteins isolated from milled rice flour. The amino acid compositions of the RP fractions of 7%-DM bran are presented in Table 3. Albumin, globulin, and glutelin contained 52.12%, 58.68%, and 36.86% total amino acids (TAAs) by weight, respectively, whereas the protein isolated from milled rice had 84.60% TAAs. Kalman [29] reported the TAA content of both the brown rice concentrate and isolate to be approximately 78% by weight. The milled rice flour represented more than 80% of the TAA values, whereas the various RBPs had low TAA values, with that for the glutelin fraction in particular being the lowest.

All the proteins analyzed in this study contained more than 35% of eight of the nine essential amino acids (EAAs), with tryptophan being the one absent. Proteins isolated from brown rice contain 37% EAAs. The EAA values for the protein fractions and milled rice flour in this study were similar to those from a previous study. Kalman [29] reported that the amino acid content and composition of a RP isolate and concentrate extracted from organic brown rice were similar to those of a soy protein isolate, but the TAA and EAA levels were lower than those of a whey protein isolate.

The milled rice flour protein, consisting of more than 70% glutelin, had the highest content of glutamic acid, followed by arginine and aspartic acid. By contrast, the cystine and methionine contents were the lowest (< 2000 mg/100 g), and the threonine, histidine, and lysine contents were also low. The albumin, globulin, and glutelin fractions isolated from 7%-DM bran had similar amino acid patterns to the milled rice flour protein. They also contained relatively higher amounts of glutamic acid, aspartic acid, and arginine and lower contents of cystine and methionine. However, among the four RP fractions, albumin had the highest content of lysine, the first limiting amino acid of grain. In fact, one of the reasons for the high nutritional value of bran is its high lysine content, which is due to its higher albumin content in RBP than in REP, which was confirmed in the current study. Likewise, Amagliani et al. [16] reported that rice bran contained a higher concentration of lysine than milled rice flour, which was attributed to the higher proportion of albumin in rice bran [16, 28, 30]. In our study, globulin contained a higher proportion of histidine and arginine than other amino acids. However, there was no difference in the amino acid

Table 3 Amino acid composition of protein fractions isolated from rice bran prepared with 7% degrees of milling

	Albumin		Globulin		Glutelin		Milled rice	
	mg/100 g	%Total AA	mg/100 g	%Total AA	mg/100 g	%Total AA	mg/100 g	%Total AA
Aspartic acid	4931.63	9.46	4740.53	8.08	3066.19	8.32	7702.23	9.10
Threonine	2752.76	5.28	1847.88	3.15	1463.77	3.97	2982.95	3.53
Serine	2591.87	4.97	2831.12	4.82	1800.18	4.88	4509.14	5.33
Glutamic acid	7890.77	15.14	9935.09	16.93	5370.17	14.57	15,357.17	18.2
Proline	2929.06	5.62	2519.33	4.29	1770.77	4.80	3713.14	4.39
Glycine	3447.24	6.61	3353.84	5.72	2192.96	5.95	3774.46	4.46
Alanine	4003.12	7.68	3259.24	5.55	2401.15	6.51	4590.50	5.43
Cystine	1244.91	2.39	887.52	1.51	462.23	1.25	960.60	1.14
Valine	2744.30	5.27	3189.47	5.44	2140.46	5.81	4894.98	5.79
Methionine	1004.19	1.93	1012.27	1.73	837.13	2.27	1713.88	2.03
Isoleucine	1702.77	3.27	2070.17	3.53	1375.86	3.73	3591.15	4.24
Leucine	2909.48	5.58	3766.00	6.42	2855.24	7.75	7165.59	8.47
Tyrosine	1724.79	3.31	2139.87	3.65	1425.58	3.87	4910.07	5.80
Phenylalanine	1522.04	2.92	2849.76	4.86	1898.87	5.15	5182.95	6.13
Histidine	1921.77	3.69	3163.07	5.39	1649.08	4.47	2317.91	2.74
Lysine	3892.54	7.47	3282.14	5.59	2077.07	5.63	3214.15	3.69
Arginine	4907.80	9.42	7833.32	13.35	4076.62	11.06	8108.14	9.58
Total amino acid	52,121.04		58,680.60		36,863.34		84,598.99	

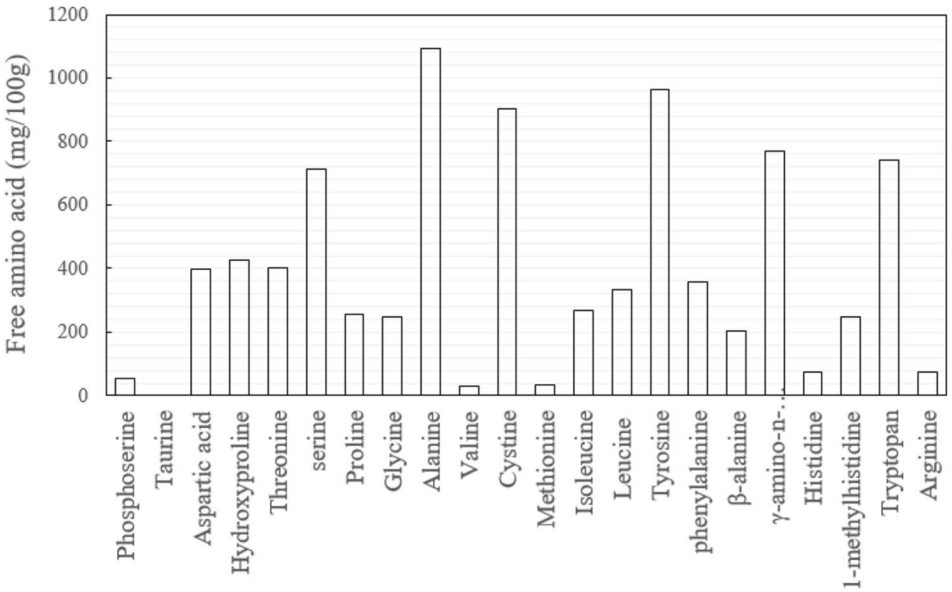

Fig. 2 Free amino acids detected in albumin isolated from rice bran prepared with 7% degrees of milling

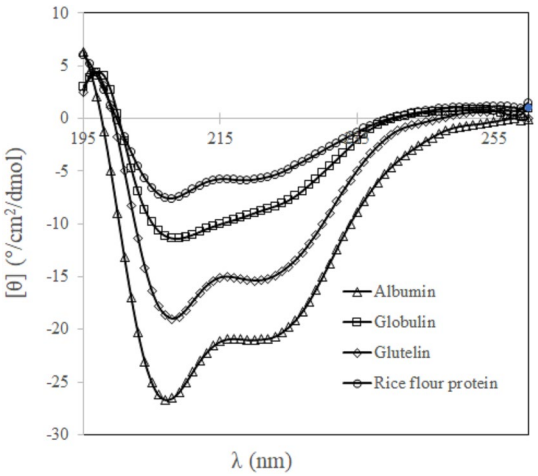

Fig. 3 Circular dichroism spectra of milled rice flour protein and three rice protein fractions

composition depending on the degree of milling. According to Krishnan et al. [31], although the sulfur-rich globulins contain no lysine, they do possess moderately high amounts of cystine and methionine, which was different from our findings.

The free amino acids detected in albumin are shown in Fig. 2. Phosphoserine, aspartic acid, hydroxyproline, threonine, serine, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, β-alanine, γ-amino-*n*-butyric acid, histidine, 1-methylhistidine, tryptophan, and arginine were detected. The levels of alanine, cystine, and tyrosine were the highest, followed by γ-amino-*n*-butyric acid, tryptophan, and serine.

Circular dichroism spectral analysis of the secondary structures of the bran proteins

The secondary structures of albumin, globulin, and glutelin measured using CD spectroscopy are shown in Fig. 3. The three RP fractions had similar peak patterns in their CD spectra, showing a positive peak at 195 nm accompanied by two negative peaks at 210 and 220 nm, which are characteristic of a secondary structure rich in α-helices. Compared with that of the other protein fractions, the peak intensity of albumin at 210 and 220 nm was larger, suggesting that it had a more stable α-helical structure. Using Fourier-transform infrared (FTIR) spectroscopy, Adebiyi et al. [7] determined the secondary structures of the four RP fractions isolated from de-oiled rice bran. According to their report, globulin had an unordered random coil configuration with an antiparallel chain of intramolecularly bonded β-sheets, whereas albumin had an ordered α-helical conformation with intermolecularly bonded β-sheets. They suggested that these structures may be the reason for the difference in solubility between albumin and globulin. They also reported that glutelin had a disordered random coil configuration with a strong antiparallel chain of β-sheets.

Previous studies have shown that the structures of the RBP fractions consist of an α-helix, a β-sheet, and a random coil. In the present study, the CD spectral results indicated that the α-helical structure was the dominant structural characteristic of the four RP fractions. In another FTIR spectroscopic study, rice globulin was suggested to possess a high α-helical content with large quantities of β-sheets and β-turns, and the conformation was influenced by various buffer environments and heat treatments [32].

Conclusions

The various RP fractions in rice bran were obtained through a sequential extraction process in a sodium chloride solution, water, an alcohol solution, an alkali solution, and an acetone solution, and the proportions of water-soluble protein fractions were compared among the 3%-, 5%-, and 7%-DM bran samples. The three types of bran showed different chemical components and populations of the four RP fractions. The 7% DM of brown rice is the stage at which the bran layers are completely removed and white rice is produced. Therefore, the 7%-DM bran had a relatively higher starch content and lower protein content than the 3%- and 5%-DM brans. In the 7%-DM bran, the content of water-soluble RP fractions (e.g., albumin) was the highest, suggesting that the sub-aleurone layer surrounding the endosperm, which was expected to be included in this bran sample, contained more albumin than the other bran samples. Our study suggests that the bran by-product remaining after a relatively high DM of brown rice to produce white rice has a high yield of water-soluble RPs and therefore has excellent potential to be recycled as a value-added product for commercial food applications.

Author contributions Prof MS was responsible for conceiving the idea and wrote the manuscript. Prof SM supervised the work, reviewed manuscript and was involved in finalization. Dr MB and Dr JN carried out the experiments.

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Data availability All data analysed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no conflict of interests.

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