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Assessment of antioxidant activity, amino acids, phenolic acids and functional attributes in defatted rice bran and rice bran protein concentrate

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Summary. The antioxidant capacities, amino acid composition and phenolic acid profile of defatted rice bran (DRB) and rice bran protein concentrate (RBPC) were investigated. The functional attributes of DRB and RBPC were also evaluated. The obtained results showed that DRB and RBPC owned good antioxidant activities, which give DPPH of 56.56 and 48.17%, ABTS of 45.34 and 32.27%, and a metal chelating activity of 53.14 and 58.81%, respectively. Essential amino acids in RBPC displayed a greater abundance when compared to DRB, whereas DRB had higher individual phenolic acid contents than RBPC. Furthermore, the results showed that ferulic acid was released in the highest concentration between various phenolics followed by p-coumaric acid. Also, the obtained data exhibited that RBPC had good emulsifying activity and foaming capacity than DRB. Our results indicate that DRB could be useful as natural antioxidants and can be used as an inexpensive alternative to synthetic food additives. Additionally, rice bran protein concentrate has an excellent prospective as functional food which can be effective for future food applications.

Keywords: Antioxidant activity, defatted rice bran, functional attributes, rice bran protein concentrate, essential amino acids

Introduction

Rice bran, a major by-product in the rice processing operation, contains protein (12–18%) and phytochemicals that have useful health effects. Rice bran is also abundant in dietary fiber and mineral elements (1). Furthermore, it is a suitable functional food for consumers because of its hypoallergenic and antioxidative characteristics (2, 3).

Phenolics have been widely studied due to they had a different range of bioactivities such as antioxidant (4), antimicrobial (5), antiviral (6), and overall for the improvement of human health. Rice bran is an important source of bioactive phenolics (7, 8). These phenolic properties can be applied to prevent certain chronic diseases, for example, obesity, cancer, and cardiovascular diseases (9, 10). The phenolic compounds, such as polyphenolic and flavonoids, are commonly present in whole cereal. Gallic, ferulic, caffeic, syringic, vanillic, protocatechuic, and cinnamic acids were stated as common phenolic acids in whole grain (11). In a recent report, ferulic and p-coumaric acids were detected as the most plentiful phenolic acids in the bran of most rice species (12).

The demand for inexpensive sources of proteins is growing, and many reports are conducted on diverse plant sources of proteins (13, 14). Rice bran protein (RBP) is considered necessary because of rich in essential amino acids and has a pleasant taste in comparison with other grains and beans (15). The health influences of rice bran have helped a lot of scientists to investigate its efficacy to be utilized as an essential agent of nutrients in diet constituents (16, 17). Moreover, for food applications, RBP is desired due to it has other attributes such as foaming, water/oil holding, and emulsifying abilities (15, 18). Alkali ex-

traction, followed by acid precipitation, is the most popular technique employed for the extraction of protein from cereals. Alkali splits hydrogen, amide bonds, and disulfide, resulting in a reduction in the molecular mass of protein and the increase in the extraction of protein (19). Therefore, the research aimed to prepare RBPC from defatted rice bran (DRB) by alkali extraction technique. The DRB and RBPC were assessed for their antioxidant activities, total phenolic and protein contents, emulsifying and foaming properties, and oil/water-binding capacity. Moreover, amino acid and phenolic acid profiles of these extracts were also determined. This study could support necessary information concerning the biological and physicochemical properties of such products that would serve to prepare their application in foods.

Materials and Methods

Materials

Rice bran was provided from a local market (Beijing, China). Phenolic acids (gallic acid, p-hydroxybenzoic acid, trans-cinnamic acid, ferulic acid, p-coumaric acid, caffeic acid, chlorogenic acid, vanillic acid, and syringic acid), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonate (ABTS), 1,1-diphenyl-2-picryhydrazyl (DPPH), ferrozine, and all the other reagents employed were analytical grade and purchased from Sigma chemical Co. (St. Louis, USA).

Preparation of defatted rice bran (DRB)

Rice bran de-oiled by extracting three times with hexane (1:3 w/v) in a lab mixer for 60 min at 250 rpm. After that, the sample was centrifuged at 4°C at 10,000×g for 15 min. The DRB left overnight to dry, which was later pulverized into a powder, sieved, packed, and stored at 4°C until analysis.

Preparation of rice bran protein concentrate (RBPC)

The RBPC was prepared by the alkaline extraction/isoelectric point precipitation method as described by Zaky et al. (20). The DRB sample was suspended in MilliQ water (1:5 w/v) and then agitated in a lab mixer for 5 min. To discard the insoluble elements, the pH of the slurry was adjusted to 9.0 by NaOH (1.0 N) and stirred for 2 h. Afterward, centrifugation of the slurry at 10,000×g for 15 min at 4 °C was used. Then, the pH of the sample was adjusted to 4.0 by HCl (1.0 N) and centrifuged again at the same conditions. The sample was then neutralized, lyophilized and saved at -20°C until analyses.

Protein content

The protein amount of treatments was assessed as explained previously by AOAC (21).

Total phenolic content (TPC)

The TPC of DRB and RBPC was done according to the technique outlined by El-Faham et al. (22). A 20 µL of the sample was diluted with 1.58 mL of MilliQ water, and then Folin-Ciocalteu reagent (100 µL) was mixed. After 3 min. 300 µL of 20% sodium carbonate was mixed. The solution was incubated for 30 min in the dark. The absorbance was measured at 765 nm (Cary 60 spectrophotometer, Agilent Technologies, USA). Gallic acid was used as a reference to construct a standard curve. The data were displayed as mg GAE per 100g sample.

Determination of phenolic composition

The individual phenolic compounds of extracts were evaluated using an Agilent HPLC 1260 Infinity II system (Agilent Technologies, USA). An Agilent Zorbox SB-C18 column (250 × 4.6 mm i.d., 5 µm) was used at a column temperature of 30°C. The filtered sample (20 µL) was injected and the mobile phase was consisted of 0.1% formic acid (A) and methanol (B) with a flow rate of 1.0 mL/min. The gradient was set as follows: 0 minutes 25% B; 20 minutes 25% B; 30 minutes 35% B; 40 minutes 100% B; 42 minutes 100% B; 50 minutes 25% B. The peaks of chromatogram were detected at 280 nm (23). The levels of each compound were quantified based on a standard curve, and the values were displayed as mg per 100 g DW of rice bran.

Amino acid profile

The amino acids composition of DRB and RBPC were hydrolyzed with 6N HCl at 110 for 24 hours according to Adebiyi et al. (24). Amino acids were measured by an amino acid analyzer (LA8080, Japan).

Antioxidant activity

DPPH ability

The DPPH ability was investigated based on the technique described by Wang et al. (25). Forty microliters of sample were added to 2900 µL DPPH (0.1 mM) that was suspended in 80% methanol. The solution was put in the dark for 30 min, after which the absorbance was read at 517 nm. The DPPH activity was calculated accordingly to this equation:

DPPH activity (%) = $1 - (Abs_{sample}/Abs_{control}) \times 100$.

Metal chelating capacity

The metal chelating capacity was measured as described by Oh et al. (26). Samples (1.0 mL) were combined with 0.1 mL FeCl2 (2 mM). Thereafter, 0.1 mL ferrozine (5 mM) and 3.0 mL MilliQ water were added to the reaction mixture and mixed thoroughly. The reaction mixture was then allowed to react for 10 min at ambient temperature. The absorbance was examined at 562 nm, and the metal chelating capacity was estimated using the equation:

Metal chelating ability (%) = $1 - (Abs_{sample}/Abs_{control}) \times 100$.

ABTS assay

The ABTS test was evaluated as reported by Zaky et al. (20). The ABTS mixture was produced by combining 7 mM ABTS and 2.45 mM potassium persulfate in the same amount and permitted the combination to set for 16 h in the dark. The ABTS mixture (1.0 mL) was suspended in 70 mL ethanol prior to the assay. Then 20 µL of samples were appended to the ready diluted solution (2.0 mL). After placed the samples at ambient temperature for 7 min, the absorbance of the resultant solution was controlled at 734 nm. The ABTS inhibition % was assessed as follows:

ABTS inhibition (%) = $1 - (Abs_{sample}/Abs_{control}) \times 100$.

Functional properties

Emulsifying properties

Emulsifying ability was outlined by the procedure of Esmaeili et al. (27), with minor modifications as follows: 25 mL of 0.1% (w/v) of samples solution at pH (3.0, 5.0, 7.0 and 8.0) were dispersed in soybean oil (10 mL) and homogenized in an electric homogenizer at 10,000 rpm for 3 min to obtain the emulsion. Afterward, 50 µL of the emulsion was removed from the 50-mL centrifuge tube at 0 and 10 min and diluted to 5 mL with SDS (0.1%). The absorbance was estimated directly after emulsion production at 500 nm. To calculate the emulsion stability index (ESI), the following equation was employed:

$ESI = A_0 \times t/\Delta A$

where, A_0 is the absorbance at 0 min and ΔA is the shift in absorbance transpiring across the time interval t (10 min).

Foaming capacity

The foaming ability was assessed as explained by Bandyopadhyay et al. (28) with some modifications. 20 mL of aqueous dispersions (1.0% w/v) of DRB and RBPC at pH 3.0, 5.0, 7.0 and pH 8.0 were merged in an electric homogenizer for 5 min at 10,000 rpm. To calculate the foaming capacity, the following equation was employed:

Foaming capacity (%) = Volume after mixing/Initial volume ×100

Water and oil absorption capacity

Water and oil absorption capacities of samples were conducted by AACC approved method 56-20 (29). One gram of sample was mixed with 10 ml MilliQ water or refined soybean oil, and allowed to stand at room temperature for 30 min, centrifuged at 3000×g for 20 min. Water or oil absorption capacity was displayed as percent water or oil bound per gram of the sample.

Water retention capacity (WRC)

The water retention capability of DRB and RBPC was achieved using the AACC method 56-11.02 (29). 1.0 g of the sample was immersed in 10.0 mL of MilliQ water for 60 min in a centrifuge tube. After centrifuging at 4000×g for 10 min, the supernatant was discarded. Then, the samples were placed to drain for 15 min by setting the tubes in an oven at 45°C. The sediment was weighed, and the WRC was estimated by subtracting the original sample weight.

Swelling capacity (SC)

The swelling attribute of samples was done as outlined by Raghavendra et al. (30) with minor modifica-

tions. 0.2 g of DRB and RBPC soaked in 10 mL of MilliQ water in a 15 mL graduated test tube. The samples were left to hydrate for 24 h. The volume occupied by DRB and RBPC was recorded. SC was expressed as volume attained by the swollen sample (mL) per g bran/protein.

Bulk density

Bulk density of DRB and RBPC was performed as illustrated by Monteiro and Prakash (31). Sample (50 g) was added to an empty dry graduated measuring cylinder (100 mL). The cylinder was then tapped slightly various times to a fixed volume. After that, the volume was recorded, and bulk density was calculated as follows:

Bulk density (g/mL) = $_{Sample}$ weight/$_{Sample}$ volume

Statistical analysis

All tests were accomplished in triplicate. Values were presented as means ± SD. The findings were achieved by applying SPSS 13.0 software (Chicago, IL, USA). The significant differences amongst samples were determined with a t-test ($p < 0.05$).

Results and Discussion

Protein content

The protein contents of the DRB and RBPC are given in Table 1. Although the content of protein for DRB was 15.11%, its pureness was increased by the alkaline extraction process and amounted to 72.45 % for RBPC. Our data are in line with that achieved by Zaky et al. (20) and suggested that rice bran extract might be employed as a protein source.

Total phenolic content (TPC)

The TPC of DRB and RBPC extracts is displayed in Table 1. The results exhibited that TPC of DRB was higher (1370.74 mg GAE /100 g) than that of RBPC (891.62 mg GAE /100 g) ($p < 0.05$). Shen et al. (32) stated that the TPC of black rice brans of six species varied from 841 to 1245 mg of GAE /100 g DW. These amounts of total phenolic of rice grains published in the literature were lower than those of DRB and RBPC reported in the present work. This is mainly due to the variations in cultivars and the methodology used.

Phenolic acids profile

The individual phenolic acids in the extracts of rice bran are given in Table 2. In this study, nine phenolic acids (gallic acid, p-hydroxybenzoic acid, trans-cinnamic acid, ferulic acid, p-coumaric acid, caffeic acid, chlorogenic acid, p-hydroxybenzoic acid, vanillic acid, and syringic acid) were discovered. Defatted rice bran displayed a higher content of individual phenolic acids than rice bran protein concentrate except for p-hydroxybenzoic acid and vanillic acid. The results revealed that ferulic acid was the dominant phenolic in the extracts of both DRB and RBPC. Significant differences were noted between DRB and RBPC for all obtained phenolic compounds, with no chlorogenic acid and trans-cinnamic acid detected in RBPC. On the other hand, a moderate amount of gallic acid

Table 1 Protein and total phenolic contents of DRB and RBPC

Raw material	Protein content (%)	TPC (mg GAE /100 g)
DRB	15.11 ± 0.32^{b}	1370.74 ± 0.24^{a}
RBPC	72.45 ± 0.56^{a}	891.62 ± 0.17^{b}

The values are the mean ± standard deviation of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$). ***DRB*** defatted rice bran, ***RBPC*** rice bran protein concentrate, ***TPC*** total phenolic content.

Table 2 The composition of phenolic acids in DRB and RBPC (mg/100g DW)

Phenolics	DRB	RBPC
Ferulic acid	33.51 ± 0.12^{a}	29.34 ± 0.16^{b}
p-Hydroxybenzoic acid	1.64 ± 0.09^{b}	2.08 ± 0.33^{a}
Caffeic acid	2.76 ± 0.21^{a}	1.15 ± 0.11^{b}
Trans-Cinnamic acid	3.43 ± 0.06	nd
Chlorogenic acid	1.71 ± 0.76	nd
Gallic acid	6.83 ± 0.53^{a}	5.51 ± 0.21^{b}
p-Coumaric acid	22.17 ± 0.32^{a}	19.52 ± 0.06^{b}
Vanillic acid	0.36 ± 0.44^{b}	0.54 ± 0.05^{a}
Syringic acid	0.28 ± 0.16^{a}	0.25 ± 0.81^{b}

The values are the mean ± standard deviation of three replicates. The values in the same row followed by different letters are significantly different ($p < 0.05$). ***DRB*** defatted rice bran, ***RBPC*** rice bran protein concentrate. nd: not detected

(6.85 and 5.51 mg/100g DW) was found in DRB and RBPC, respectively, while lower amounts were found for syringic acid (0.26 and 0.28 mg/100g DW, respectively). Generally, these variations in phenolic acid composition might be due to a difference in cultivars, harvesting conditions, extraction chemicals, and procedures utilized by various researchers (22, 33, 34).

Amino acid composition

The amino acid profile of DRB and RBPC is given in Table 3. Amino acid profile displayed insignificant variations in both DRB and RBPC. Tyrosine (Tyr) was the highest plentiful amino acid in all extracts, followed by Ala, Arg, Asp, and Glu, respectively. Besides, RBPC had higher amounts of total essential amino acids (35 g/100g protein) than DRB (34.39 g/100g protein). Hence, rice bran extracts not only had a high antioxidant capacity but also possessed good nutritional value. Wang et al. (17) mentioned that the major amino acids in rice bran protein were glutamic acid, aspartic acid, and arginine, respectively. As reported by Farvin et al. (35), several amino acids, such as Tyr, Arg, Thr, His, and Lys can be exerted as antioxidant abilities. Zaky et al. (36) stated that acidic amino acids could be played an essential role in the chelation of metal ions by carboxyl and amino groups in their side chains. In this study, the DRB and RBPC had two acidic amino acids, such as Asp and Glu, which accounted for 9.65, 8.11 and 9.67, 8.22% of total amino acids, respectively. Discrepancies in the amino acid composition may be attributed to the variation in rice bran cultivar utilized in this research.

Table 3 Amino acids composition of DRB and RBPC (g/100g protein)

Amino Acids	DRB	RBPC
Essential		
Histidine (His)	2.15 ± 0.12^{b}	2.21 ± 0.41^{a}
Isoleucine (Ile)	4.27 ± 0.18^{b}	4.32 ± 0.10^{a}
Leucine (Leu)	5.76 ± 0.23^{b}	5.85 ± 0.11^{a}
Lysine (Lys)	6.47 ± 0.41^{b}	6.53 ± 0.13^{a}
Phenylalanine (Phe)	5.16 ± 0.15^{b}	5.23 ± 0.32^{a}
Methionine (Met)	1.65 ± 0.09^{a}	1.67 ± 0.06^{a}
Valine (Val)	4.54 ± 0.17^{b}	4.63 ± 0.23^{a}
Threonine (Thr)	3.32 ± 0.42^{a}	3.34 ± 0.12^{a}
Tryphtophan (Trp)	1.07 ± 0.05^{b}	1.12 ± 0.13^{a}
Non-essential		
Aspartic Acid (Asp)	9.65 ± 0.24^{a}	9.67 ± 0.11^{a}
Serine (Ser)	3.26 ± 0.16^{b}	3.34 ± 0.29^{a}
Glutamic acid (Glu)	8.11 ± 0.10^{b}	8.22 ± 0.07^{a}
Cystine (Cys)	2.74 ± 0.08^{a}	2.11 ± 0.25^{b}
Alanine (Ala)	9.84 ± 0.06^{a}	9.87 ± 0.14^{a}
Arginine (Arg)	9.00 ± 0.32^{b}	9.73 ± 0.22^{a}
Glycine (Gly)	5.43 ± 0.14^{a}	5.47 ± 0.16^{a}
Proline (Pro)	2.78 ± 0.09^{a}	1.12 ± 0.23^{b}
Tyrosine (Tyr)	14.80 ± 0.18^{b}	15.57 ± 0.11^{a}

The values are the mean ± standard deviation of three replicates. The values in the same row followed by different letters are significantly different ($p < 0.05$). ***DRB*** defatted rice bran, ***RBPC*** rice bran protein concentrate.

Antioxidant activity

The antioxidant capacity of DRB and RBPC extracts was examined using DPPH, ABTS, and metal chelating activity methods. As shown in Table 4, significant differences ($p < 0.05$) were observed among DRB and RBPC extracts for all antioxidant activities assays. The DPPH scavenging activity and ABTS values of the DRB were higher than RBPC, which was consistent with the variation in the phenolic. This concept was in the same trend with Singh et al. (37), who observed a strong correlation among the TPC contents and DPPH efficacy from methanolic pomegranate peels extract. Also, the increase in the scavenging of free radicals for DRB extract may be attributed to its hydrogen donating ability. Xu and Godber (38) reported that ferulic acid showed the highest antioxidant activity of rice bran at the three different ratios in a linoleic acid model. These findings were similar to our results in terms of ferulic acid, which was a major phenolic acid among all tested samples.

On the other hand, it was observed that RBPC gave a better ability to chelate metal ions (58.81%) when

Table 4 Antioxidant activities of the DRB and RBPC (%)
The values are the mean ± standard deviation of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$). ***DRB*** defatted rice bran, ***RBPC*** rice bran protein concentrate.

Fractions	DPPH activity	Metal chelating activity	ABTS inhibition
DRB	56.56 ± 0.72^{a}	53.14 ± 0.22^{b}	45.34 ± 0.52^{a}
RBPC	48.17 ± 0.08^{b}	58.81± 0.11^{a}	32.27 ± 1.12^{b}

compared with DRB (53.14%). These may be owing to amino acid chains, such as lysine and arginine, which capable of interacting with metal ions (20). Also, our findings suggest that the observed higher antioxidant activity of these extracts might be owing to the ion chelation ability of its inherent acidic amino acids (Asp and Glu), as reported above. Generally, Rice bran is rich in phytochemicals and antioxidant factors; thus, removal of the bran layer throughout the processing of polished rice reduces the antioxidant ability (39).

Emulsifying activity

The emulsifying properties of the DRB and RBPC are shown in Fig. 1a. The different conditions of pH were observed to affect the emulsifying activities of DRB and RBPC. The emulsifying ability of the RBPC was significantly ($p < 0.05$) increased when pH transformed from acidic to neutral compared with DRB. Similar findings were stated by Hamada (40) for rice bran protein hydrolysates. This might be attributed to producing hydrolysates with high solubility, and smaller molecular masses that promote the spread rate and improve the interaction among lipid and protein (28). In this work, the use of RBPC under various pH values improved the emulsifying properties of the isolated protein.

Foaming capacity

Since the pH rose from 3.0 to 8.0, DRB and RBPC increased slowly in foaming capacity (Fig. 1b). The minimum foaming capability of DRB and RBPC was noted at pH 3.0 with values 14.50% and 43.56%, respectively. The highest foaming ability of DRB and RBPC was 20.3% and 68.6% at pH 8.0, respectively. Besides, Figure 1b showed an increased foaming capacity of RBPC over DRB at all pH values. These results coincided with previous studies and confirmed that pH value is considered one of the most critical factors affecting both volume and resistance of foams (15, 27). The isolates of rice endosperm and rice dregs-based protein possessed a foaming capacity of 116 and 119%, respectively, and foaming stability of about 98% after 20 min (41).

Water and oil absorption capacity

Water and oil absorption capability perform a crucial part in the functional features of rice bran to be used in food systems, including meat, bakery, and beverage. The findings of water and oil absorption are illustrated in Fig. 1c. The obtained data showed that RBPC has a good water absorption ability (3.85 ± 0.12 g/g) compared with DRB (1.41 ± 0.07 g/g), which indicated that RBPC could be employed in goods which need abundant water absorption. Proteins with high water absorption aid to minimize moisture loss in patisserie. Furthermore, it is desired to keep the sweetness and wet mouth-feel of baked products (42). The obtained data in this study are consistent with those informed by Esmaeili et al. (27), where RBPC exhib-

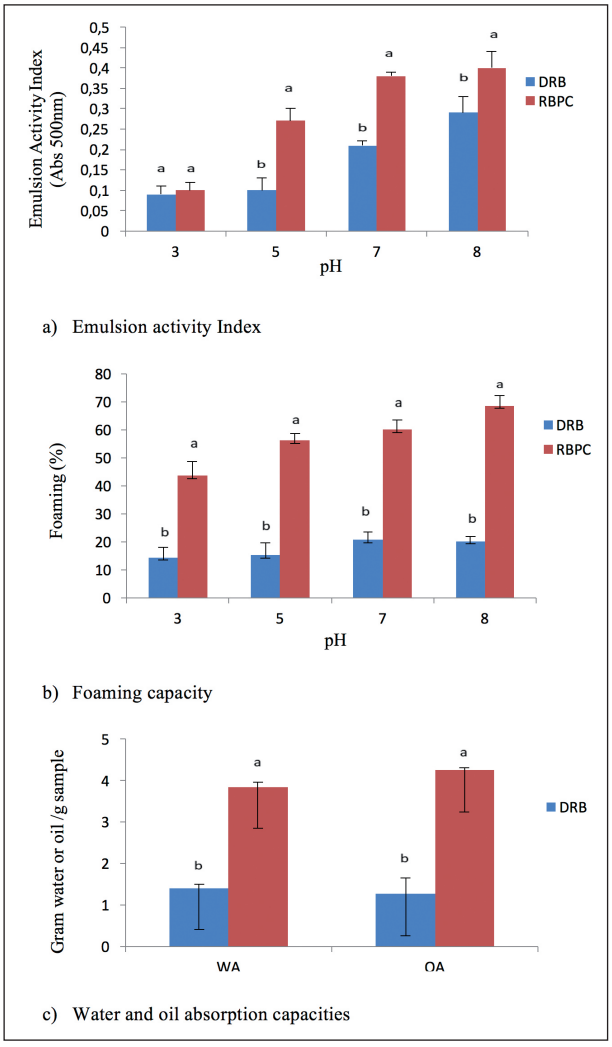

Figure. 1 Functional properties of defatted rice bran (DRB) and rice bran protein concentrate (RBPC). a) Emulsion activity Index, b) Foaming capacity, c) Water and oil absorption capacities. WA water absorption, OA oil absorption. Bars with different letters have significantly different ($p < 0.05$) mean values. The values are mean ± SD of three replicates.

ited high water absorption capacity due to the high crude fiber content. Cao et al. (43) informed that the water absorption capacity of rice bran protein (3.54 mL/g) was higher than that of proteins released from brown (1.96 mL/g) and white rice (1.78 mL/g).

High oil absorption directly influences the food systems formulation, for example, cake batters and sausages. Data indicated that the oil absorption of RBPC was significantly better than that of DRB ($p < 0.05$). This may be owing to higher levels of nonpolar amino acid side chains that bind the hydrocarbon side chain of oil. Also, the low hydrophobicity of DRB could not help the interaction within proteins and fat, leading to a reduction in oil absorption ability (27). Our results revealed that the oil absorption of RBPC was 4.25 ± 0.10 g/g, which was greater than of rice protein (0.87 g/g) and whey protein (0.74 g/g), which found by Esmaeili et al. (27). In the work of Zhao et al. (41), protein isolates produced from rice endosperm and rice dregs owned an oil absorption capacity of 2.14 and 2.38 g/g, respectively.

Water retention capacity

Water retention ability is an indicator of the efficacy of food product flour to absorb the water and can be used as an index of gelatinization. It is highly affected by the starch content as well as that of protein and fiber (44). In the present study, RBPC was found to be having more considerable water retention ability compared to DRB as shown in Table 5. The higher value of water retention capacity in the gelatinized RBPC was because of the existence of undamaged long polymer chains. A comparable trend was perceived by Jacobs et al. (45) for wheat bran.

Table 5 Water retention capacity, swelling capacity and bulk density of DRB and RBPC

Raw material	Water retention capacity (g/g)	Swelling capacity (ml/g)	Bulk density (g/ml)
DRB	1.77 ± 0.02[b]	7.0 ± 0.07[b]	0.52 ± 0.006[a]
RBPC	2.16 ± 0. 01[a]	12.5 ± 0.07[a]	0.35 ± 0.005[b]

The values are the mean ± standard deviation of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$). ***DRB*** defatted rice bran, ***RBPC*** rice bran protein concentrate.

Swelling capacity

The swelling capacity of DRB and RBPC is provided in Table 5. The results exhibited that RBPC has much swelling capacity (12.5 ± 0.07 ml/g) compared with DRB (7.0 ± 0.07 ml/g). Swelling capacity is considered as a feature criterion in some suitable formulations such as bakery goods. The swelling ability of bran depends on the mass of particles, kinds of varieties, and types of processing techniques or unit procedures (46). According to Chandra et al. (46), parboiled rice flour has a higher swelling capacity than raw rice. They also reported that the swelling capacity of composite flours was risen with an increase in the level of incorporation proportion of rice, green gram, and potato flour and reduced with wheat flour level.

Bulk density

Bulk density is an essential agent in preparing of food packaging. It is commonly affected by particle size and flour density (44). Bulk density amounts for DRB and RBPC were 0.52 and 0.35 g/ml, respectively (Table 5). These data were comparable to the values (0.55 and 0.53 g/ml) reported by Esmaeili et al. (27) for Tarom and Shiroodi cultivars, respectively. Moreover, the bulk density of DRB and RBPC obtained in this study was higher than that of Basmati 386 RBP (0.12 g/ml), whereas they are less than casein (0.89 g/ml) (42). The decline in bulk density of RBPC could be a benefit when utilized in the development of weaning food formulations where lower bulk density is desired. Previous studies showed that the bulk density of bran can be associated with the degree of milling and is found to be significantly improved as the degree of milling increased (47).

Conclusion

This study exhibited that RBPC can be isolated from DRB by alkali extraction. The RBPC had higher essential amino acid content, water retention and swelling capacities, and enhanced functional properties than DRB. Whereas, DRB exhibited better antioxidant abilities and individual phenolic acid contents than RBPC. The present investigation provided valuable knowledge about rice bran products for more

significant employment in food applications as natural antioxidants and functional components with desirable emulsifying and foaming attributes, as well as appropriate for infant formulas.

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