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Antinutritional Appraisal and Protein Extraction from Differently Stabilized Rice Bran

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Abstract: Rice bran from 'Basmati Super' cultivar was stabilized by dry heat, microwave heat and parboiling. All the stabilization techniques were found effective in reduction of antinutrients including trypsin inhibitor, haemagglutinin-lectin and phytates. No adverse effect of stabilization was observed on chemical composition of rice bran. Protein was extracted from differently stabilized rice bran along with unstabilized bran by enzymatic extraction. Protein isolates yield remained highest for the unstabilized bran, followed by Microwave Stabilized Rice Bran Protein Isolates (MWRBPI), Dry Heat Stabilized Rice Bran Protein Isolates (DHRBPI) and Parboiled Rice Bran Protein Isolates (PAR-RBPI).

Key words: Stabilization, isolates, protein recovery, yield

INTRODUCTION

Rice bran is a co-product obtained from rice milling processing; composed of several botanical entities including pericarp, nucleus, aleuron and subaleuron layers, seed coat and some endospermic portion. It constitutes about 10% of the weight of rough rice (Hu *et al*., 1996). It is comprised of pericarp, aleurone, sub-aleurone, seed coat, nucellus along with the germ and a small portion of endosperm (Salunkhe *et al*., 1992; Hargrove, 1994). Rice bran is a rich source of protein with dual benefits of being plenteous and economical (Shih, 2003).

Rice bran is prone to a number of physicochemical changes soon after its detachment from kernel. For long term storage pre-treatments are necessary to either cease or slow down the detrimental changes. The term stabilization owes to pretreatment of bran prior to protein extraction to check the quality deteriorative elements. It aims at inactivation of lipases, principally responsible for lipid deterioration during storage, hydrolysis of phytic acid into myo-inositol and available phosphorous and to denature the antinutritional factors, including trypsin inhibitor and haemagglutinin lectin proteins, to reduce their toxicity without affecting quality of bran protein. Thermal processing denatures the naturally occurring antinutritional factors and thus has widely been used for reduction of antinutrients in the plant based foods and thus increases the nutritional value of native and isolated protein products (Wiseman and Price, 1987; Urbano *et al*., 1995; Seena *et al*., 2006).

Protein isolates can be prepared from a number of cereal by-products. Several processing techniques are in practice for isolation of protein from parent material. The fundamental aspect of these techniques is solubilization of protein in the extractant and then subsequent removal and purification by appropriate method. Bran proteins have a strong aggregation tendency due to extensive disulfide bonding; makes the proteins hard to extract (Hamada, 1997). Therefore, physical, chemical, enzymatic agents are employed to facilitate the extraction process. Rice bran and its extracts have found an increased potential for food application in recent times. Rice bran protein has hypocholesterolemic activity and potency to fight against cancer and tumor formation (Kawamura *et al*., 1993; Helm and Burks, 1996; Han *et al*., 2001).

MATERIALS AND METHODS

Rice bran from freshly milled cultivar "Basmati Super" was procured from Reem Rice Mills (Pvt.) Muridke. Cereal flours (wheat, maize and rice) and other ingredients for weaning food preparation were purchased from local market, Faisalabad. Enzymes and reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Rice bran stabilization: The stabilization process was carried out to make a halt to lipid matrix degeneration process, which starts soon after bran detachment from kernel. To achieve the objective rice bran was subjected to various stabilization techniques. Raw rice bran without any stabilization treatment was used as controlled Unstabilized Rice Bran Sample (Un-RB) to evaluate the effect of heat treatment on bran.

Dry heat stabilized bran (DH-RB): For dry heat stabilization rice bran was placed uniformly in 0.5 cm layer in pre-cleaned drying oven tray and placed in pre-heated oven (DO-1-30/02, PCSIR, Pakistan) at 120°C for 10 min, followed by cooling at ambient temperature (Sharma *et al*., 2004).

Microwave stabilized rice bran (MW-RB): Added moisture technology was applied for rice bran stabilization through microwave oven (OM8035-M, Orient, Japan). Rice bran moisture content was first raised upto 21%, One hundred gram of sample was packed in a microwave-safe polyethylene bag and spread out in 0.5 cm layer, placed on microwave resistant glass tray (6 inch dia.) and subjected to microwave heating for 3 min at 100% power and then cooled at room temperature (Ramezanzadeh *et al*., 2000).

Parboiled rice bran (PAR-RB): Paddy of Basmati Super was soaked for 8 h at 60°C, steaming at 6.5 PSI at 120°C for 10 min in autoclave (MLS-3780-SV, Sanyo) followed by drying in aluminum trays (10x15 inch) at ambient temperature to a final 12-13% moisture content (Biswas and Juliano, 1988). Bran was obtained from parboiled paddy using commercial milling facility.

Bran storage: All bran samples were packed in zipper-top bags and stored at room 5°C till further use.

Rice bran anti-nutrients: Anti-nutrients pose risk associated with cereals and cereal based products as with rice bran. The bran samples were uniformly mixed with 20% (w/w) solution of 1% calcium hydroxide to analyze the anti-nutrient peril lying with stabilized and unstabilized rice brans. Analyses were carried out for haemagglutinin-lectin activity, trypsin inhibitor and phytates as follows:

Haemagglutinin-lectin activity: Haemagglutinin activity of raw and processed rice bran samples was determined by Rabbit Erythrocyte Agglutination Test (Benedito-de and Barber, 1978). Lectin activity was measured in Haemagglutinin Units (HU) as reported by Tan *et al*. (1983).

Trypsin inhibitor activity: For the purpose, bran sample was blended with 0.05N HCl in a Sorvall Omni Mixer (Ivan Sorvall, Inc., Newtown). The extracted slurry was centrifuged and Trichloroacetic Acid (TCA) was added to the supernatant and re-centrifuged. After neutralization the enzyme inhibitory activities were determined as described by Decker (1977).

Phytates: Phytic acid content of raw and processed rice bran samples was determined by following the method of Haug and Lantzesch (1983). Samples were heated with acidic ammonium iron-III sulphate solution of known concentration. The decrease in iron content (determined colorimetry with 2,2 bipyridine at 519 nm) spectrophotometer (CE 7200-7000 series, Cecil, UK) in supernatant was the measure of phytate content.

Rice bran defatting: The bran samples were defatted using 1:3 bran to solvent ratio together with shaking in 500 mL conical flask, thrice volume of hexane to bran, together with shaking at 250 rpm in orbital shaker (DO20206, Sanyo, UK) for 30 min followed by centrifugation at 4000xg (M-3K30, Sigma, Germany). The defatted rice bran was air-dried for eight hours and stored at 5°C in zipper top bags.

Raw materials analysis

Proximate analysis: Rice bran samples were analyzed on dry weight basis for crude protein, crude fat, crude fiber, ash and Nitrogen Free Extract (NFE) according to their respective methods by AACC, 2000. Nitrogen content in each sample was determined by using Kjeltech Apparatus (Technik GmbH D-40599, Behr Labor, Germany) based on Kjeldhal's AACC Method 46-10, protein percentage was calculated by multiplying with conversion factor 5.95. Crude fat content of each rice bran sample was estimated by using Soxtech System (HT2 1045 Extraction Unit, Hoganas, Sweden) by following the AACC method 30-10. Crude fiber content of each rice bran sample was determined by digesting the sample in 1.25% H_2SO_4 and 1.25% NaOH solution through Labconco Fibertech (Labconco Corporation, Kansas, USA) as described in AACC Method 32-10. Ash content in each dry sample was determined by incinerating 3 g sample in a Muffle Furnace (MF-1/02, PCSIR, Pakistan) after charring according to AACC Method 08-01. NFE content was calculated by difference method.

Preparation of rice bran protein isolate (RBPI): Protein isolates were prepared from defatted rice bran samples described by Wang *et al*., 1999. The slurry prepared from defatted rice bran was subjected to enzymatic treatment with xylanse (250units/g rice bran) and phytase (350phytase units/g rice bran) followed by incubation for 2 h. After incubation period, enzyme activity was halted by pH alteration of medium coupled with continuous stirring for half an hour. The whole slurry was then centrifuged at 10,000xg and supernatant was separated (M-3K30, Sigma, Germany). The pH of supernatant was adjusted to 4.0 with 0.1 N HCL solution. Precipitated proteins were separated from the medium by centrifugation (10,000xg), followed by neutralization and freeze drying. The protein isolates thus prepared were stored in moisture resistant polyethylene bags at 5°C.

Protein recovery: Recovery of rice bran protein isolates was expressed as weight of protein isolates, obtained after enzymatic extraction process, per 100 g weight of parent bran.

Protein yield: Protein yield was calculated as a ratio of weight and protein percentage of protein isolate to weight and protein percentage of its respective type of

bran (Wang *et al*., 1999) to quantify the final product as a measure of variability in stabilization techniques.

Statistical analysis: Data was obtained by applying Completely Randomized Design (CRD) and the results were analyzed through analysis of variance technique (Steel *et al*., 1997) using Cohort version 6.1 (Co-stat 2003) to determine the level of significance. The separation of means or significant difference comparisons were done using Tukey's HSD test and DMRt.

RESULTS AND DISCUSSION

Chemical composition: Significant variations were observed among differently stabilized rice bran samples for crude fiber and NFE content whereas crude protein, crude fat and ash content showed non-significant differences. Results for proximate composition (Table 1) indicated that defatted rice bran samples contain 18.16±0.72 to 19.05±0.39% crude protein, 0.54±0.10-0.57±0.06% crude fat, 11.67±0.47 to 17.74±0.66% crude fiber, 9.51±0.60 to 10.15±0.50% ash content and 52.51±0.98 to 60.01±0.84% nitrogen free extract, on dry weight basis. The results showed significantly higher crude fiber and NFE contents in parboiled rice bran. This was might be due to starch gelatinization during parboiling process resulting in high fiber content (Kahlon and Smith, 2004).

Similar results have been observed by Saunder (1990) for protein (15-20%), fat (0.5-1.5%), ash (9-12%) and fiber content (10-15%). In another study, rice bran samples were found to contain 13.2-17.3% protein, 3.2-9.5% crude fiber and 9.2-11% ash content (Pomernaz and Oryl, 1982). Likewise, 15.32 and 17.72% protein and 11.87 and 12.74% ash content were found in defatted unstabilized and stabilized rice bran on wet basis (Gnanasambandam and Hetiarachchy, 1995). The present results of parboiled rice bran were in conformity with the findings of Sekhon *et al*. (1997) who found 19.1% crude protein, 10.6% crude fiber and 11.9% of ash content. Chemical composition rice bran obtained from Pakistani rice cultivars contain 6.68% moisture, 15.78% crude protein, 20.55% crude fat, 7.59% crude fiber and 41.51% NFE (Sharif *et al*., 2005).

The means for the crude protein content of different rice bran samples revealed highest value in PAR-RB (19.05±0.39%) followed by Un-RB (18.25±0.51%), MW-RB (18.17±0.16%) and DH-RB (18.16±0.72%). These results revealed that the heat stabilization treatments did not affect the crude protein content. The results are in conformity with the findings of Ramezanzadeh *et al*. (2000), who found non-significant differences in chemical composition of microwave stabilized rice bran. Similarly, microwave cooking did not affect the proximate composition of legumes (Naveeda and Prakash, 2004). The highest fiber content was observed in PAR-RB (17.74±0.66%) which was significantly different from the other three brans including DH-RB (11.90±0.87%), MW-RB (11.85±0.48%) and Un-RB (11.67±0.47%). In an earlier study, high fiber content was noted in parboiled rice bran (14.5%) as compared to defatted unstabilized bran (10.5%) (Prakash, 1996).

Antinutritional factors

Phytates: Interaction of phytates with metals results in insoluble complexes resulting lower absorption and impaired solubility of protein (Bera and Mukherjee, 1989; Abdelrahaman *et al*., 2007). Stabilization treatment significantly reduced the phytate content. Maximum phytate content was present in unstabilized rice bran (4.01%); heat stabilization reduced the phytate content of rice due to inactivation of phytates (Table 2). The lower mean values for phytates highlighted the importance of stabilization. Maximum reduction of phytate content (0.85%) was observed in parboiled bran which had followed by microwave stabilized bran (1.07%) and dry heat stabilized bran (1.28%). Both dry and moist heat are capable of reducing the phytic acid content of rice bran resulting in improved nutritional quality of bran (Takemasa and Hijikuro, 1991; Sharma *et al*., 2004; Tangendjaja *et al*., 2006).

Haemagglutinin-lectin: Heat labile haemagglutinin is growth depressant at lower level in foods and tends to be toxic at high concentration (Liener, 1994). The haemagglutinin, rice bran lectin, is capable of binding to specific carbohydrate receptor sites in intestinal wall thereby lowering the nutrient absorption (Goldstein *et al*., 1980). The haemagglutinin-lectin activity showed decline after stabilization (Table 2).

Unstabilized rice bran exhibited the maximum activity (22.58 activity/mg), while reduction was achieved in dry heat stabilized (1.14), microwave stabilized (0.92) and parboiled rice bran with minimum activity (0.18). The results of present investigation are in accordance with the other researchers who accomplished the inactivation of the haemagglutinin-lectin activity heating the bran upto 100°C (Sayre *et al*., 1987; Rehman and Mahmood, 1996). Thermal processing of rice bran inactivates the toxic haemagglutinin in rice bran (Benedito and Barber, 1978). Microwave heat treatment significantly reduces haemagglutinin-lectin activity in beans and legumes (Hernández -Infante *et al*., 1998).

Trypsin inhibitor: Trypsin inhibitors are responsible for impairing trypsin enzyme activity in the digestive tract (Bahnassey *et al*., 1986). These can be inactivated at elevated temperature processing (Liener, 1994). The results of trypsin inhibitor activity explicated the significance of stabilization techniques. Trypsin inhibitor activity can be used as a possible indicator of processing to obtain the protein isolates hypoallergenic

Table 1: Means for Proximate composition of defatted rice bran samples (%dry weight basis)

Rice Bran	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	NFE (%)
Un-RB	18.25±0.51	0.56±0.12	11.67±0.47	9.51±0.60	60.01±0.84[a]
MW-RB	18.17±0.16	0.57±0.06	11.85±0.48	9.54±0.76	59.86±0.42[a]
DH-RB	18.16±0.72	0.54±0.10	11.90±0.87	9.63±0.72	59.77±0.57[a]
PAR-RB	19.05±0.39	0.54±0.10	17.74±0.66	10.15±0.50	52.51±0.98[b]

Superscripts indicate the application of DMR, Means sharing the same letter in a column are not significantly different

Un-RB-Unstabilized rice bran MW-RB-Microwave stabilized rice bran

DH-RB-Dry heat stabilized rice bran PAR-RB-Parboiled rice bran

Table 2: Means for antinutritional factors of rice bran samples

Rice bran	Phytates (%)	Haemagglutinin-lectin activity/mg	Trypsin inhibitor activity/mg
Un-RB	4.01±0.40[a]	22.58±0.75[a]	8.44±0.51[a]
MW-RB	1.07±0.14[b]	0.92±0.19[bc]	Nil
DH-RB	1.28±0.24[b]	1.14±0.25[b]	Nil
PAR-RB	0.85±0.09[b]	0.18±0.03[c]	Nil

properties. The highest activity was observed in unstabilized rice bran (8.44 activity/mg), whereas all three stabilized brans showed no detectable activity of trypsin inhibitors.

Bera and Mukherjee (1988) found the residual trypsin inhibitor activity of 6.03 TIU/mg of unstabilized bran protein extracts. Heat treatment readily inactivates trypsin inhibitor in rice bran (Tashiro and Ikegame, 1996). Microwave treatment, dry and moist heat treatment are capable of marked reduction in trypsin inhibitors (Deolankar and Singh, 1979; Friedman and Brandon, 2001; Hernandez-Infante *et al*. (1998).

Rice bran protein isolates: Recovery and yield: Significant variations were observed among stabilized and unstabilized rice bran protein isolates. The mean values for protein isolate recovery and yield (Table 3) were affected by preliminary rice bran stabilization techniques.

Rice bran is a rich source of quality protein generally isolated by wet alkaline extraction or with the aid of physical processing or enzymatic treatment (Tang *et al*., 2002; Tang *et al*., 2003). The protein recovery differed significantly among various protein isolates. The results pertaining to the protein recovery in terms of total weight of protein isolates obtained from bran showed significant differences between the stabilized and unstabilized protein isolates. Highest recovery (18.32±0.29 g/100 g bran) was noted in PAR-RBPI had highest recovery followed by MW-RBPI (17.73±0.54 g/100 g bran) and DH-RBPI (17.67±0.36 g/100 g bran). The overall recovery of protein isolates ranged from 16.84-18.32%.

It is obvious from the results (Table 3) that the highest protein yield (78.90±3.93%) was obtained from Un-RBPI followed by MW-RBPI (69.61±1.83%) and DH-RBPI (67.59±4.65%), while the lowest yield (58.75.±1.49%) was in PAR-RBPI. The overall protein yield for protein isolates ranged from 58.75±1.49 to 78.90±3.93%.

The most common method for protein extraction from rice bran is alkaline extraction method to solubilize the protein and subsequent precipitation to from protein products (Chandi and Sogi, 2007). Enzymatic processing has introduced novelty to protein extraction process to produce food grade protein isolates (Hamada, 2000). Enzymatic hydrolysis of proteins not only improves the protein yield but also the immunological and functional attributes and widens the span protein isolates application in foods (Mannheim and Cheryan, 1990).

Table 3: Means for proximate composition of RBPI

Protein Isolate	Protein recovery (g/100g bran)	Protein yield (% bran protein)
Un-RBPI	16.84±0.22[b]	78.90±3.93[a]
MW-RBPI	17.73±0.54[a]	69.61±1.83[b]
DH-RBPI	17.67±0.36[a]	67.59±4.65[b]
PAR-RBPI	18.32±0.29[a]	58.75±1.49[c]

Un-RBPI-Unstabilized rice bran protein isolates

MW-RBPI-Microwave stabilized rice bran protein isolates

DH-RBPI-Dry heat stabilized rice bran protein isolates

PAR-RBPI-Parboiled rice bran protein isolates

Protein recovery and yield of isolates varied within the differently stabilized and unstabilized bran used in current study. The highest protein yield was obtained for Un-RBPI, PAR-RBPI showed the highest protein recovery from bran in terms of weight but with the lowest protein content (Table 3) of parboiled rice bran protein isolates resulted in the lowest protein yield of PAR-RBPI, this might be due to degree of denaturation of protein, which affected the extract ability and contamination of non-protein components of bran like carbohydrates. Similar but less intensive tendency was observed for both MW-RBPI and DH-RBPI samples, results for protein recovery of these two isolates remained non-significant with PAR-RBPI but differed significantly from Un-RBPI.

Wang *et al*. (1999) and Xu *et al*. (2006) could obtain protein yield from 34.2±1.1 to 74.6±4.1% and still higher (86%) by combination of different enzymes from unstabilized bran. In a similar study, higher recovery of soluble proteins (81.4 and 87.6%) was observed from rice bran (Hamada, 2000). Likewise, 3.7-72.4% and 4.7-

72.2% protein could be recovered from unstabilized and stabilized rice bran, respectively varying with the particle size of bran (Gnanasambandam and Hetiarachchy, 1995). However, physically processed rice bran can yield 65.5-80.6% and 62.2-87.1% residue protein (Anderson and Guraya, 2001). L'hocine *et al.* (2006) could recover 46.0-78.6% protein in isolates from differently processed soy meals.

Conclusion: Stabilization did not affect the chemical composition of rice bran, while parboiled rice bran had slightly higher protein, fiber and ash content. Heat labile anti-nutrients were efficiently reduced by all the three stabilization techniques i.e. parboiling, dry heat and microwave treatment; together with variably influenced the protein recovery and yield. Parboiling might have resulted in some textural modifications that reduce the final yield of protein isolates. Recovery remained highest (18.32%) for parboiled rice bran protein isolates but the yield, in terms of extracted protein content, remained lowest (58.75%).

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