RICE BRAN PROTEIN CONCENTRATES OBTAINED BY WET ALKALINE EXTRACTION[1]

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ABSTRACT

Protein concentrates were extracted from full-fat rice bran with dilute sodium hydroxide at 24° C, followed by separation of the fibrous residue, and heat or acid precipitation of the extracted protein. In an alternate procedure, the starch fraction was separated prior to protein precipitation. Protein concentrates containing 23–31% protein, 33–48% fat, and 15–23% starch were obtained in yields of 14–20%. Protein concentrates with the bulk of the starch removed contained 33–38% protein and 49–55% fat. Fats were 86% unsaturated. Protein efficiency ratio and nitrogen digestibility of the concentrates were significantly greater than those of the starting bran. Nitrogen solubility curves, amino acid profiles, fatty acid composition, and mineral content of the concentrates are reported, as well as yield and composition of by-products produced during the process. Alkaline extraction of commercially defatted rice bran is also reported.

Rice bran, a by-product of the milling of brown rice to produce white rice, consists of the outer bran layers of the kernel and part of the germ, and constitutes 8.8 to 11.5% by weight of brown rice (1) and about 8% of rough rice (2). Rice protein, though limiting in lysine and threonine, has one of the highest nutritive values among cereal proteins (1), and the protein content of the bran is higher than any other portion of the rice kernel (3). In addition to protein, the bran fraction is rich in fat, starch, free sugars, B vitamins, and minerals (1). However, factors such as high fiber content, contamination with hull fragments, and the rapid development of rancidity and free fatty acids upon storage have restricted the use of rice bran largely to animal feed or fuel. The major portion is used as an animal feed (2). A small percentage is used as human food in the form of brown rice. In some countries, rice bran is used almost exclusively as fertilizer or fuel (2).

Methods have been presented describing the alkaline extraction of protein from defatted rice bran (4,5) and full-fat rice bran (6). The optimum extraction conditions suggested were pH 11 for 1–2 hr (4) and 0.05% sodium hydroxide (20 vol) for 2 hr at 37° C (5). A high-protein concentrate has been prepared from X-M defatted bran[2] by a process consisting of starch gelatinization, treatment with a diastatic enzyme, and a two-stage alkali extraction of protein followed by acid precipitation (7).

This paper describes the preparation of a high-protein, low-fiber concentrate by mild alkaline extraction of rice bran. The process works equally well with bran which is rancid and/or adulterated with hulls. It also uses simpler, more economical, and less severe conditions than previous processes and has been adapted to pilot plant operation. Yields, composition, and some properties

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Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[2]Brown rice pretreated with steam and rice oil is milled in the presence of a rice oil/hexane miscella to give white rice, defatted bran, and rice oil as separate products.

TABLE I
Yield and Composition of Protein Concentrates and By-Products Obtained by Processing of Rice Bran[a] at pH 9

Processing	Yield	Protein	Fat	Fiber	Ash	Starch	Phosphorus	Phytate Phosphorus
Concentrates								
Heat ppt[b]	20.5	22.8	32.7	0.7	11.7	22.9	...	...
Heat ppt	17.5	24.7	36.4	1.1	13.5	17.0	2.85	2.49
Heat ppt[c]	14.2	29.1	42.3	0.6	9.8	14.9	...	...
Acid ppt	16.8	28.2	41.7	1.0	5.2	20.4	1.08	0.59
Acid ppt[c]	13.8	31.3	47.7	0.7	5.8	16.1	...	...
Starch removed, heat ppt	12.2	33.3	49.2	0.5	4.5	1.3	...	...
Starch removed, heat ppt[c]	11.3	34.0	54.7	0.2	1.9	0.8	...	...
Starch removed, acid ppt	12.3	35.8	50.8	0.6	3.3	1.5	0.80	0.41
Starch removed, acid ppt[c]	12.0	37.9	54.2	0.3	2.3	0.2	...	...
By-products								
Pressed residue	67.6	8.8	5.9	21.2	10.2	31.5	...	...
Starch fraction	5.6	3.0	1.7	1.9	42.9[d]	35.2	11.0	8.77
Clear juice[e]	14.4	7.3	...	...	19.6	...	...	...

[a]Stored bran except where noted (b); laboratory scale preparations.
[b]Freshly milled bran.
[c]Washed.
[d]Ash constituents accounted for as % of ash: 24.1% phosphorus, 9.7% potassium, 9.2% magnesium, 4.9% silica, 4.8% sodium, 1.4% calcium, and 0.9% carbonate.
[e]Sugar content, 52.1% (determined as an equimolar mixture of glucose and fructose).

(physical, nutritional, and functional) of the protein concentrates are described.

MATERIALS AND METHODS

Rice bran[3] from the commercial milling of Calrose rice was donated by the Grosjean Company, San Francisco. Defatted rice bran, commercially defatted by the X-M process, was donated by Riviana Foods, Inc., Abbeville, La. Proximate analyses calculated on a moisture-free basis were: rice bran: 2.01% N, 13.74% fat, 14.45% fiber, 12.06% ash, and 25.4% starch; defatted rice bran: 3.08% N, 5.36% fat, 8.55% fiber, 11.24% ash, and 31.6% starch.

Laboratory Preparation of Protein Concentrate

Rice bran protein concentrates were prepared as follows: the rice bran (100 g) was mixed with 5 vol of water, the pH was adjusted to 9 with dilute sodium hydroxide, and the mixture was stirred for 15 min at room temperature (24° C). The mixture was hydraulically pressed at 40 psi to separate the alkaline pressed juice from the fibrous residue, and the protein was precipitated by heat (steam injection to 85°C at pH 6) or acid (pH 4). The protein concentrates were separated from the supernatant 'clear juice' by centrifugation (3050 × *g* for 10 min) and dried by lyophilization. In some instances, the products were washed prior to lyophilization by resuspending in water acidified with dilute HCl (pH 4 for acid precipitate and pH 6 for heat precipitate). To assess recycling of the extractant, the clear juice (adjusted to 5 vol with water and pH 9 with sodium hydroxide) was used as the extraction solvent of the next batch of bran.

[3]Rice bran refers to the full-fat bran, unless otherwise noted.

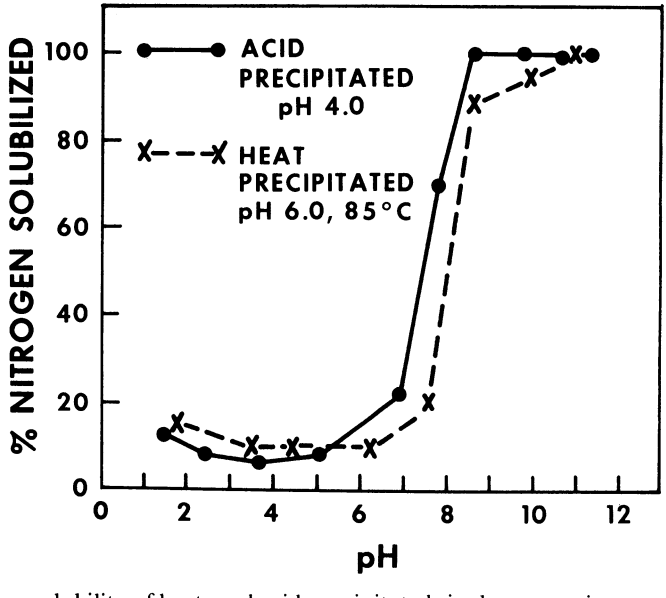

Fig. 1. Nitrogen solubility of heat- and acid-precipitated rice bran protein concentrates as a function of pH.

Low-starch precipitates were prepared by centrifugation of the alkaline pressed juice (1465 × *g* for 5 min) and separation of the starch fraction prior to heat or acid precipitation of the protein. The starch fraction was washed with water prior to drying by lyophilization.

Pilot Scale Preparation of Protein Concentrate

Forty pounds of rice bran was mixed with 200 lb water and enough 3*N* NaOH to maintain the mixture at pH 8.6–9.0 (about 4.4 lb) using an air-driven Lightnin® mixer (Model A1100). Portions of the slurry (one-fourth at a time) were placed in a nylon press bag, covered with oak press boards, and hydraulically pressed in a cider press at pressures gradually increasing to 44 psig. The alkaline juice was adjusted to pH 5.8 with 3*N* HCl and divided; half was steam-injected to 85° C, and the other half was acidified to pH 4.2. The heat- and acid-precipitated concentrates were collected by centrifugation at 1500 rpm, using a size 17 Fletcher Standard Centrifuge, and lyophilized at 25° C in a modified Stokes vacuum oven.

Feeding Study

Protein efficiency ratios (PERs) were determined on the pilot plant preparations by the AOAC method (8) using groups of five rats for each diet. All diets were adjusted to 10% protein. A test diet of casein supplemented with 14% corn oil was included. PERs were adjusted to 2.50 for the casein control diet.

Solubility Profiles

The protein solubility profiles of the rice bran protein concentrates, with and without the starch fraction, were evaluated in 1% solutions (w/v) over the pH range of 1.5 to 11, according to the procedure of Betschart (9).

Analytical

Crude protein was determined as 5.95 × Kjeldahl nitrogen. Fat, nitrogen, ash, and fiber were determined by AOAC methods (8), starch by pig α-amylase digestion (10), minerals by X-ray fluorescence spectroscopy (11), phytic acid by the method of Wheeler and Ferrel (12), total solubles after enzymatic digestion (TSAE) by the method of Guggolz *et al.* (13), and sugars by the method of Saunders and Walker (14). Color was measured on a Hunter D-25 color difference meter with Hunter lab standard D33C-157 as the reference color.

Amino acid analyses were made with an automatic amino acid analyzer using the modification and correction factors described by Kohler and Palter (15). The sulfur amino acids were determined after oxidation with performic acid. Fatty acids were determined by gas chromatography of the methyl esters (16).

RESULTS AND DISCUSSION

In the laboratory, products containing 23–31% protein, 33–48% fat, and 15–23% starch were obtained in yields of 14–20% by wet processing at pH 9 and 24° C (Table I). Where starch was removed during the process, yields of the concentrates were lowered to 11–12%; however, the protein and fat contents increased to 33–38% and 49–55%, respectively. Acid-precipitated concentrates exhibited higher protein, fat, and starch contents, and lower fiber and ash than

those precipitated by heat, when starch was retained in the product. Washing the products prior to drying lowered yields but increased the levels of protein and fat compared to unwashed products.

The product from stored bran extracted at pH 9 and heat precipitated (Table I) showed a decrease in yield over that prepared under similar conditions from freshly milled bran (line 2 *vs.* line 1, Table I). Though the bran was stored at 0° C over the 1-year period, rancidity as judged by odor was noted, and this may be a factor which lowered the protein yield. For experimental purposes, it was considered necessary to maintain a single source of bran in order to have one source of material, even though it was realized that protein quality and extractability could possibly change with time. After the 1-year period, twice as much alkali was required to maintain the extraction pH at 9 in the case of stored bran; this may be due to the formation of free fatty acids during storage. Other investigators have found that in milled rice, interaction of proteins with the breakdown products of lipid oxidation decreased alkali-soluble protein (17).

To decrease water required for processing, and drying costs required to recover the solubles in the clear juice as a concentrate, a recycle experiment was carried out in which the clear juice (supernatant above the protein precipitate) from a prior extraction (after adjustment to 5:1, solvent:bran ratio with water) was used as the extraction solvent of the following run. No experimental difficulties were encountered during a ten-batch recycle operation. The percentage of solids in recycled juice leveled off at cycle 5. The yield and protein content of the products obtained did not change as the clear juice was recycled; however, the fat content continuously decreased. A formula was developed to calculate the amount of water required during a recycle operation: $y = x(1.97n + 4.35)$, where y equals the weight of water, x equals the weight of rice bran, and n equals the recycle batch number. In the ten cycles carried out extracting 100 g of bran at a time, an average of 75 g of water, 17 g of 3*N* NaOH, 23 g of 1*N* HCl, and 92 g of steam were used per cycle. The y value includes the weight of water from all these sources. This recycle operation was carried out on stored bran; the amount of NaOH required to adjust the mix to pH 9 would have been halved if fresh bran had been used.

TABLE II
Protein Efficiency Ratio and Nitrogen Digestibility of Rice Bran[a] and Protein Concentrates Prepared by Wet Alkaline Processing of Rice Bran[a]

Dietary Source of Protein	PER[b,c]	% N Digestibility[d]
Casein control	2.50a	100.1
Rice bran	1.59c	58.5
Acid-precipitated protein concentrate[e]	2.19b	89.6
Heat-precipitated protein concentrate[f]	1.99b	83.4
Casein plus 14% fat	2.50a	100.9

[a]Stored bran. Pilot plant preparations.
[b]PER values were adjusted to a casein control value of 2.50.
[c]PER values bearing a different letter notation are significantly different; P = 0.01 (Duncan's test).
[d]Corrected for metabolic nitrogen excretion.
[e]Composition: 25.3% protein, 36.4% fat, 2.0% fiber, 7.4% ash, and 20.1% starch.
[f]Composition: 23.4% protein, 32.8% fat, 2.9% fiber, 14.9% ash, and 14.8% starch.

PER and nitrogen digestibility were determined for rice bran and heat- and acid-precipitated protein concentrates prepared in a pilot scale operation. The results are shown in Table II. A test diet of casein plus added fat (14%) was included to determine whether a high fat content comparable to the test protein concentrates would affect PER. No such effect was observed. Although final mean body weights of rats on control and test diets did not differ significantly, the PERs of both the acid- and heat-precipitated concentrates were significantly greater than that of rice bran. The increased PER is presumably due to the higher nitrogen digestibility and lysine content of the concentrates compared to the bran. Mitsuda *et al.* (5) found the digestibility of rice bran and rice bran protein isolate to be 71 and 94%, respectively, as compared with casein (100%) after an *in vitro* pepsin digestion.

The amino acid patterns of rice bran samples used in the feeding study are shown in Table III. The lysine content is greater in the concentrates than in the starting bran. Mitsuda *et al.* (5) also found a similar difference. Using the 1973 FAO provisional amino acid scoring pattern (18), the first limiting amino acid of rice bran was lysine, followed by isoleucine. The chemical score was 80.2. The first and second limiting acids of the concentrates were threonine and isoleucine, respectively. Chemical scores for the heat- and acid-precipitated concentrates were 88.5 and 90.5, respectively.

TABLE III
Amino Acid Content of Rice Bran and Protein Concentrates Prepared by Extraction of Rice Bran at pH 9[a] (g AA/16 g N)

Amino Acid	1973 FAO Provisional Amino Acid Scoring Pattern	Rice Bran	Concentrate	
			Heat-precipitated	Acid-precipitated
Lysine	5.44	4.41[b]	5.19	5.08
Histidine	...	2.42	2.92	2.97
Ammonia	...	1.92	1.49	1.49
Arginine	...	7.19	8.80	8.69
Aspartic acid	...	9.56	8.50	8.15
Threonine	4.0	3.69	3.54[b]	3.62[b]
Serine	...	4.52	4.64	4.55
Glutamic acid	...	13.09	13.62	14.16
Proline	...	3.79	4.58	3.66
Glycine	...	5.27	5.71	5.54
Alanine	...	6.18	6.23	6.00
Valine	5.0	5.85	6.29	6.09
Isoleucine	4.0	3.53[c]	3.69[c]	3.72[c]
Leucine	7.0	6.72	6.97	6.84
Tyrosine	6.0	2.54	3.27	3.04
Phenylalanine		4.20	4.64	4.15
Cystine	3.5	2.24	1.72	1.82
Methionine		1.83	1.76	1.66
Nitrogen recovery (%)		85.65	90.68	88.47

[a]Pilot plant preparations.
[b]First limiting amino acid.
[c]Second limiting amino acid.

The heat- and acid-precipitated concentrates were found to have the same fatty acid composition: oleic (48.9%), linoleic (36.8%), palmitic (14.1%), linolenic (trace), and stearic (trace). This accounted for 97% of the fatty acids; of this, 86% was unsaturated. No change in the fatty acid pattern was observed after storage of the concentrates at 37°C for 4 months.

The concentrates contained 300–400 ppm of iron, making them a potentially good dietary source of iron. Copper and zinc contents in all the concentrates were lower than in original brans.

Ultimately, it is expected that these protein concentrates would find use as food for humans and, thus, they should possess desirable functional as well as nutritional properties. One possible food use would be incorporation into a protein-rich milk-like beverage; accordingly the nitrogen solubility profiles of the heat- and acid-precipitated concentrates were determined and are shown in Fig. 1. Analysis (TCA) of the solubilized nitrogen in the supernatants indicated that 80–85% was protein nitrogen. The solubility of the acid-precipitated concentrates increased at pH 7 and above. At pH 8, 80% was soluble and at pH 8.4 and above, 100% was soluble. The heat-precipitated concentrate was less soluble at pH 7 and 8, but was increasingly soluble at 8.5 and above. Other heat-precipitated preparations showed considerable variability in the shape of the solubility curve for reasons presently unknown. Both concentrates showed minimum solubility (6 to 15%) between pH 1.5 and 6.5.

The concentrates were a light tan color; this may be due to residual bran pigment or browning by amino acid-sugar interactions. The acid-precipitated concentrates were slightly darker than those precipitated by heat.

The yield and composition of the by-products obtained during processing are shown in Table I. Evaluation of the pressed residue as a potential animal feed indicated that this by-product could be used as a feed for ruminants. An *in vitro* enzymatic digestion procedure, which measures solubilized solids (TSAE) and which has been correlated with total digestible nutrients in alfalfa and cereals (13), was used to evaluate feed potential. Because the residue contained starch, an additional enzymatic digestion with α-amylase was also used. The TSAE, and TSAE plus α-amylase, values for the pressed residue were 46.8 and 51.3,

TABLE IV
Yield and Composition of Protein Concentrates and By-Products Obtained by Processing of Commercially Defatted Rice Bran at pH 9

Sample	Yield %	Protein %	Fat %	Fiber %	Ash %	Starch %
Defatted rice bran, X-M process	...	18.3	5.4	8.6	11.2	31.6
Heat-precipitated concentrate	20.5	33.7	8.2	1.6	17.0	25.5
Acid-precipitated concentrate	16.8	43.0	12.2	1.6	5.7	29.7
Heat-precipitated, starch removed	9.3	58.2	16.2	1.0	6.1	3.2
Acid-precipitated, starch removed	11.0	62.3	17.7	0.6	3.4	1.6
Starch	10.9	3.8	0.4	1.8	37.0	39.9
Pressed residue	63.2	14.1	4.1	13.0	9.3	36.6

respectively. This compares with a TSAE of 50.4 for dehydrated alfalfa (15% protein), indicating that the pressed residue would be suitable in ruminant feed formulations.

The composition of the clear juice left after precipitation of the concentrate was examined to determine potential feed value. Though high in ash (19.6%), this fraction contained 7.3% protein and 52.1% sugars and, thus, after concentration might have value as a feed molasses. The sugars were tentatively identified by paper and thin-layer chromatography to be sucrose, glucose, fructose, and raffinose. Glycerol was also present in the juice. Rice bran sugars had been previously (19) identified as sucrose and raffinose.

The starch fraction, isolated by centrifuging the alkaline extract prior to heat or acid precipitation of the concentrate, contained only 35.2% starch, the major portion being ash (42.9%). Analysis of the ash showed that it contained 24.1% phosphorus, 80.9% of which was present as phytate phosphorus.

Concentrates from Defatted Bran

Protein concentrates were prepared by wet alkaline extraction of rice bran which had first undergone commercial defatting by the X-M process. The conditions of processing were the same as for full-fat bran, except that the solvent-to-bran ratio was 4.5:1. Yields and composition of the concentrates and by-products are shown in Table IV. The concentrates contained 34–43% protein which represents 38–40% of the starting protein, 8–12% fat, and 25–30% starch; where the starch fraction was removed prior to heat or acid precipitation, the concentrates contained 58–62% protein and 16–18% fat. The concentrates were light tan in color and developed no rancidity, as judged by odor after storage for several months at room temperature. Mitsuda *et al.* (5), using an alkaline extraction followed by acid precipitation and alcohol treatment, obtained an isolate containing 94–99% protein which represented a recovery of 30–40% of starting protein. Other workers (4,7) obtained concentrates with protein contents of 75–85% by extraction at pH 11. Evaluation of the pressed residue indicated that it had potential value as a ruminant feed.

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