Solubilization and Recovery of Protein from Defatted Rice Bran[1]

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ABSTRACT

Alkaline extraction of defatted California rice bran removed 33.3 to 82.5% of total crude protein as pH increased from 7 to 12 when the bran was stirred 1 hr. at 25°C. with 7.5 vols. of extractant (optimal conditions). Grinding the bran had little effect, though solubilization of protein and total solids increased somewhat at and above pH 10. Much of the extracted protein was separated by isoelectric precipitation (pH 5.5); a further small amount was coagulated by heating. About 16 to 17% of total crude protein remained soluble after isoelectric precipitation; about half the nitrogen in this fraction was dialyzable. At pH 11, which was optimal, neutralization and drying of the extract yielded about 50% of the bran protein as a 40% concentrate. Drying of the separated solids precipitated at pH 5.5 from the pH 11 extract yielded about 37% of the bran protein as an 85% concentrate. Gel electrophoresis showed multiple components in both soluble and precipitated fractions of the extracted protein.

Rice bran, now used as animal feed, contains considerable protein that has good qualities for human nutrition. Representative U.S. brans contain 10 to 17% crude protein (1); this is probably also about the range for world rice brans. The preparation from these brans of protein concentrates such as are now being made from various oilseeds could add a considerable amount of valuable protein to the diets of the world. This would be especially true in rice-growing regions, which also generally need increased protein food supplies.

Relatively few studies have been made that aid in evaluating rice bran proteins as foods. Kik (2,3) reported protein efficiency ratios (PER) for bran of 1.61 and 1.92 at 8.16 and 9.0% protein, respectively. Recently reported amino acid compositions of crude protein in U.S. brans (1) showed an average of 4.81 g. lysine per 16.0 g. N. This was 37% higher than the average of 3.51 g. per 16.0 g. N in milled rice protein, where lysine is the first limiting amino acid. Earlier amino acid analyses were also discussed in this article. Chakrabarti (4) has pointed out earlier studies showing rice bran to be a good source of protein, fat, minerals, and B-vitamins and summarized his work on the nutritive qualities of a fiber-free concentrate prepared from rice bran. This concentrate, containing 5.2% protein, was suggested as a food supplement with emphasis on vitamin and mineral contents.

Regular rice bran becomes rancid quickly on storage. Removal of the oil not only stabilizes the bran, but provides a source of high-quality food oil (5). The defatted bran at the same time permits an improved extraction of proteins.

Rice proteins are less soluble than those from many other grains. Although Cagampang and co-workers (6) have shown that alkaline solutions are among the most effective extractants for rice proteins, information on the use of such solutions for extracting protein from rice bran seems unavailable. Studies reported here were undertaken to determine the feasibility of producing a protein concentrate from defatted rice bran by alkaline extraction. A somewhat analogous study has been reported for wheat bran by Fellers and co-workers (7).

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MATERIALS AND METHODS

Bran

Bran from California Pearl rice (Caloro or Colusa variety) taken directly from the milling line was stored at −10°C. A stock supply was defatted by percolation with ethyl ether, and the dry, solvent-free bran was held at 4°C. This defatted bran contained 10.41% crude protein (N × 5.95), 0.69% fat, 7.94% ash, and 11.11% crude fiber, all dry basis (8).

To obtain finely ground material, defatted bran was passed once through a Mikro-Sample mill (hammer mill) with circular 0.039-in. (∿ 1 mm.) screen perforations. Particle size distributions of samples were obtained by shaking the material on U.S. Standard Sieves for 10 min. in a Ro-Tap sieve shaker and weighing the resulting fractions.

Protein and Solids Solubilization

The effect of pH (7.0 to 12.0) on the solubilization of protein (Kjeldahl N × 5.95) and solids of the brans was determined by extraction with aqueous alkaline solutions with stirring for 1 hr. at 25°C. and 7.5:1 solvent-to-bran ratio. The pH was adjusted with sodium hydroxide. Additional sodium hydroxide was added as required to maintain constant pH during extraction. Separations were achieved by centrifugation at 750 × g for 30 min. The effects of variation in extraction time from 0.25 to 4 hr., and of solvent-to-bran ratios of 5 to 15, were measured at pH 7.0 to determine the above optimum extraction conditions.

Isoelectric precipitation of protein from alkaline extracts was made by adjusting the pH with hydrochloric acid to pH 5.5 and centrifuging. Coagulated protein was obtained by gently boiling the supernatant solution from the isoelectric precipitation for 5 min., cooling, and filtering.

Protein Recovery

In recovery experiments, extractions were made under the fixed conditions described. However, the residual bran after centrifugation was washed with a volume of water equal to one-half that of the original solvent, and again centrifuged. The two supernatant solutions were combined. Coagulated protein was not determined, but was included with soluble protein remaining after isoelectric precipitation.

Analytical

Crude protein was determined as 5.95 × Kjeldahl N. Fat, ash, and crude fiber were determined by AOAC methods (8).

Electrophoresis

Electrophoresis was performed in a 6% polyacrylamide gel in a horizontal apparatus (E.C. Co.) with 0.017M aluminum lactate buffer at pH 3.2. Gels were stained with Amido Black 10B.

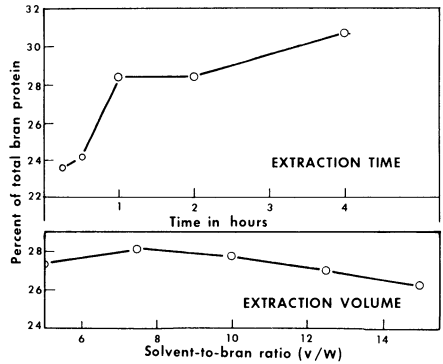

Fig. 1. Relation of extraction time and solvent ratio to protein extraction.

RESULTS AND DISCUSSION

Extraction Time and Solvent Volume

Figure 1 shows the variation in amounts of crude protein extracted with changes in time of extraction and in solvent-to-bran ratio at pH 7.0. A minimum extraction time of 1 hr. was required. Protein extracted in this time was nearly the same as in 2 hr. and 92% of that extracted in 4 hr. Hence, 1 hr. was selected as a standard time.

Maximum protein extraction was obtained with a 7.5:1 solvent-to-bran ratio. With increasingly lower ratios the slurry became too thick for effective handling. Decreased extraction at higher ratios may have reflected a dilution of the naturally occurring salts of bran in the extracting solution. The lowest effective volume would, of course, reduce the amounts of liquid to be handled.

Effect of pH

Increases in pH increased the percentage of total crude protein extracted (Fig. 2) in 1 hr. at 25°C. over the pH range investigated. The augmented yields

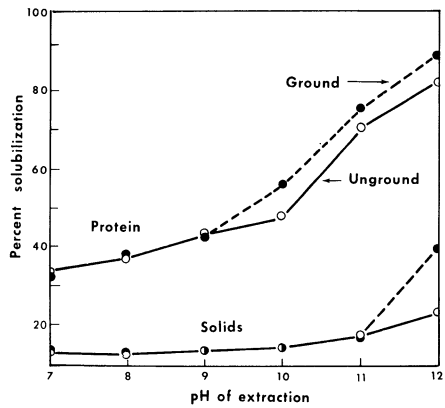

Fig. 2. Relation of pH of extraction to the protein and total solids extracted from regular and ground bran in 1 hr. at 7.5:1 solvent ratio.

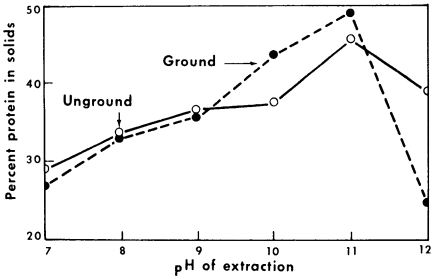

Fig. 3. Protein content of solids extracted from regular and ground brans at various pH values in 1 hr. at 7.5:1 solvent ratio.

shown for pH's above 10 indicate that some type of protein became much more soluble at the higher alkalinity. This may have been due to its intrinsic properties or to environmental conditions in the bran, such as cellular location of the protein or its binding to other constituents.

The percentage of solids extracted, which included the proteins, gradually became greater as the pH was raised, but there was no major increase in any pH range. The percentage of protein in total extracted solids of regular bran increased from 28.9% at pH 7.0 to 45.5% at pH 11 and then decreased to 38.8% at pH 12 (Fig. 3). This decrease at pH 12 resulted from the larger amount of nonprotein solids dissolved. The trend with ground bran was similar but more marked.

Grinding

The defatted regular bran contained a considerable proportion of fine-particle material (Table I), but the grinding resulted in a much finer particle size for the total sample. Grinding the bran increased solubilization of protein only above pH 9 (Fig. 2). This was in the range in which an apparent separate type of protein became soluble. The additional protein dissolved was not great and probably would not justify the grinding.

An increase in solids dissolved as the result of grinding the bran occurred only at pH 12. The undissolved solids at this pH were rather gelatinous and were difficult to separate by centrifuging. Conditions at pH 12 were generally unfavorable for protein recovery.

TABLE I. PARTICLE SIZES OF RICE BRANS

U.S. Sieve Mesh Size	Original Bran	Ground Bran
	%	%
On 18	5	...
On 40	30	...
On 60	26	7
On 80	17	11
On 100	5.5	10
Through 100	16.5	...
On 140	...	11
On 200	...	10
Through 200	...	50

TABLE II. MAINTENANCE OF EXTRACT ALKALINITY

pH	Sodium Hydroxide	
	Original	Ground
	mg./g. bran (dry basis)	
7	1.03	0.58
8	2.98	2.80
9	5.30	5.41
10	9.72	9.86
11	20.28	19.00
12	36.83	33.26

Grinding the bran had no marked effect on the amount of alkali needed to maintain alkalinity of the extracts (Table II). The logarithm of the alkali required to maintain pH of the suspension during extraction (in mg. NaOH per g. bran) had a very high correlation (r = 0.999**) with pH for both regular and ground bran between pH 8.0 and pH 12.0. Thus there is a strong inverse correspondence between alkali required to maintain pH and the hydrogen ion activity of the extracting solvent.

Protein Characterization

In Fig. 4 the protein of regular bran is classified with respect to solubility, precipitation at pH 5.5, and heat-coagulation of the protein remaining after precipitation. Protein solubility (top curve) was determined experimentally under the fixed conditions previously described. Protein precipitated at pH 5.5 (lowest curve) and by heat-coagulation (center curve minus lowest curve) was determined from experimental data corrected to 100% recovery of the solubilized protein. Thus all values in Fig. 4 represent maximum possible yields of these various fractions.

The maximum percentage yield of isoelectrically precipitated protein (lowest curve) from regular bran differed from the total dispersed protein (top curve) by 16 to 17% except at pH 12 where the difference was 23% (Fig. 3). Ground bran

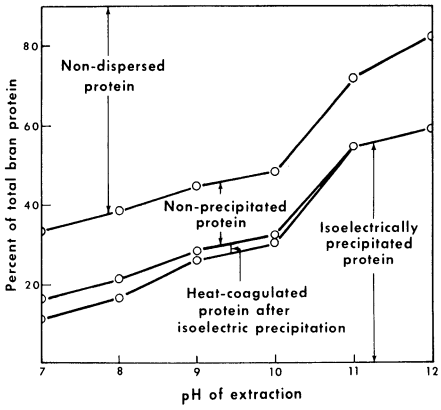

Fig. 4. Classification of total protein of regular bran with respect to solubility, isoelectric precipitation (pH 5.5), and heat-coagulation at various pH values. Extraction for 1 hr. at 7.5:1 solvent ratio.

TABLE III. CRUDE PROTEIN RECOVERY FROM RICE BRAN
(as percent of total protein)

Material	pH 9	pH 11
	%	%
Total protein	37.4	50.1
pH 5.5 ppt.	20.2	36.8
Supernatant	17.0	11.9

showed about the same difference except that it was 28% at pH 12. This relationship showed that the alkaline treatment had practically no effect below pH 12 on the isoelectric precipitation of the protein. Dialysis of the crude protein (N X 5.95) remaining soluble at pH 5.5 caused loss of about 50% of the N from the pH 9 extraction and about 40% from the pH 11 extraction. Thus about 8 to 10% of the total crude protein of bran remained as soluble nondialyzable protein. The results from ground bran were very similar.

The maximum amount of heat-coagulated protein (center minus lower curve, Fig. 4) obtainable from the pH 5.5 solution after precipitation ranged from 4.0 at pH 7 to 0 at pH 11 and above. This indicated that most of the heat-coagulated protein is extracted at neutral pH and the alkaline treatment results in a modification of those proteins that renders them less coagulable. However, it is possible that they come down in the isoelectric precipitate after alkaline modification.

Protein Recovery

Recoveries of extracted protein at pH 9.0 and 11.0 included the water-wash following separation of the extract as previously described. Total percentages of recovered protein were determined by neutralizing and freeze-drying one portion of the combined extract and wash (Table III). Isoelectrically precipitated and soluble proteins were obtained separately in the other portion. At pH 9.0 the extracted protein was separated into 54.0% precipitable and 45.5% soluble proteins; at pH 11.0 the corresponding fractions were 73.5 and 23.8% respectively.

Protein Composition

The composition (dry basis) of the products recovered at pH 9.0 and 11.0 (Table IV) differed chiefly in the larger percentages of protein and ash found at the higher pH and the correspondingly lower content of carbohydrate. Separation into precipitated and soluble fractions concentrated most of the protein and fat in the

TABLE IV. COMPOSITION OF SOLIDS FROM RICE BRAN EXTRACTS

Component	pH 9			pH 11		
	Total Extracted Solids	In ppt.	In Supernatant	Total Extracted Solids	In ppt.	In Supernatant
	%, Dry Basis					
Protein	35.6	80.4	19.5	40.7	85.0	17.0
Fat	0.5	1.1	0.2	0.5	1.3	0.3
Ash	12.4	5.4	15.3	18.5	4.2	21.7
Carbohydrate (by difference)	51.5	13.1	65.0	40.3	9.5	61.0

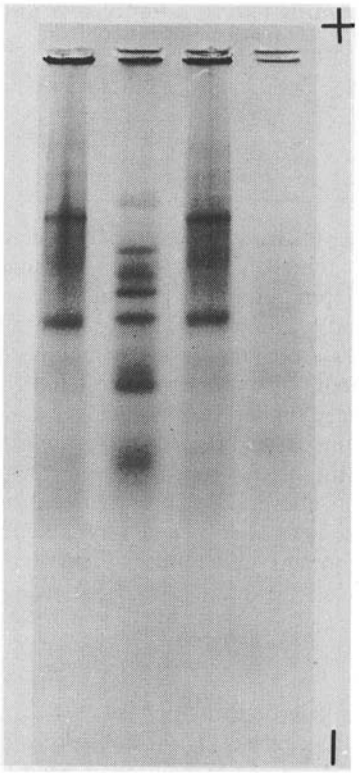

Fig. 5. Polyacrylamide gel electrophoresis patterns of protein extracted from rice bran. Run in 0.017M aluminum lactate, pH 3.2, 2 hr. at 11 v./cm. in 6% gel. From top: soluble (0.5% concentration), precipitated (1%), soluble (1%), and precipitated (2%).

precipitate and left most of the carbohydrate and ash in the soluble portion. The electrophoretic patterns (Fig. 5) obtained from the precipitated and soluble proteins illustrate clearly that each contains several components and that these components are distinctively different in the two products.

The light tan color of the dried total extracted protein may be due to presence of some bran pigment, but may also result in part from browning by amino acid-sugar interactions. Separation into the two solubility fractions and drying resulted in a very light tan precipitate and a somewhat darker tan soluble protein fraction.

CONCLUSIONS

The data presented show that extraction at pH 11 followed by neutralization and drying to residual solids will recover from a 10%-protein bran about 50% of the total protein as a 40% concentrate. By the additional step of recovering the solids precipitated on acidifying to pH 5.5, an 85% protein concentrate can be obtained corresponding to 37% of the original protein. This modified process would avoid the need for evaporating considerable amounts of liquid, and would change the resulting protein composition somewhat. Pilot-scale studies are needed to evaluate

the process, as are amino acid analyses or feeding studies for assessing the nutritional quality of the product.

Acknowledgment

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Literature Cited

1. HOUSTON, D. F., ALLIS, MARIAN E., and KOHLER, G. O. Amino acid composition of rice and rice by-products. Cereal Chem. 48: 527 (1969).
2. KIK, M. C. Nutrients in rice bran and rice polish and improvement of protein quality with amino acids. J. Agr. Food Chem. 4: 170 (1956).
3. KIK, M. C. Nutritional improvement of rice diets and effect of rice on nutritive value of other foodstuffs. Arkansas Agr. Expt. Sta. Bull. 698, Fayetteville, Ark. (1967).
4. CHAKRABARTI, C. H. Rice polishings concentrate in nutrition. J. Nutr. Dietet. (India) 4: 251 (1967).
5. HOGAN, J. T. Rice bran, oil and wax, in rice by-products utilization. Food Agr. Organ. U.N. FAO Agr. Eng. Informal Working Bull. 30, pp. 1-6 (1967).
6. CAGAMPANG, GLORIA B., CRUZ, L. J., ESPIRITU, S. G., SANTIAGO, R. G., and JULIANO, B. O. Studies on the extraction and composition of rice proteins. Cereal Chem. 43: 145 (1966).
7. FELLERS, D. A., SINKEY, V., SHEPHERD, A. D., and PENCE, J. W. Solubilization and recovery of protein from wheat millfeeds. Cereal Chem. 43: 1 (1966).
8. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis (10th ed.). The Association: Washington, D.C. (1965).

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