Effect of Microwave and Enzymatic Treatment on the Recovery of Protein from Indian Defatted Rice Bran Meal

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Abstract: Defatted rice bran meal is an important source of protein along with other micronutrients. Rice bran meal has been utilized to produce protein isolates for potential application in various food products. Attempt has been made to increase the protein solubility by physical means like microwave digestion as well as by microwave digestion followed by homogenization. Simple 40 sec microwave treatment could give the protein recovery of 78.4% as against 28.9% after 1 min of conventional boiling and 40 sec is taken as optimum time for microwave treatment. The protein recovery is further increased by microwave treatment followed by homogenization. Defatted rice bran meal has also been treated with enzyme papain and viscozyme separately to increase the protein solubility. The yield of protein isolate (RPI), prepared by alkaline extraction followed by acidic precipitation is 10.2%, which is further increased to 14.5 & 22.4% by papain, viscozyme modification and 21.1 & 22.3% by microwave treatment and microwave treatment followed by homogenization respectively. A maximum of 82.5 and 82.6% protein has been recovered as soluble protein from de-oiled bran by viscozyme treatment and by 40 sec microwave treatment followed by 10 min of homogenization. So, microwave treatment along with homogenization is a suitable alternative processes in extracting protein from rice bran meal.

Key words: rice bran meal, papain, viscozyme, protein isolate

1 INTRODUCTION

Rice bran with 12-20% protein content may be a potential source of inexpensive high quality protein for new food uses¹. The potential food uses of defatted clean bran have been reviewed by Houston². Rice bran protein containing 3-4% lysine may be an inexpensive high quality protein for food uses³. Protein has been extracted from rice bran for various food and pharmaceutical applications⁴⁻⁸. Anderson *et al.*⁷ have extracted defatted, full fat stabilized and full fat un-stabilized rice bran protein by colloid milling and homogenization. It has been observed that full fat stabilized rice bran showed breakdown in size distribution of protein after colloid milling and homogenization. Rice bran protein has been used as hypoallergenic milk replacers in infant formulas⁴. Other uses include preparation of protein isolates and concentrates from rice bran meal⁹. Relatively pure rice bran protein can be produced by alkaline extraction followed by precipitation of protein by adjusting the pH to its isoelectric point¹⁰. But the procedure for extracting protein from rice bran must be carefully selected to

produce protein isolates or concentrates with desirable functional properties⁵. Due to strong disulfide bonding and aggregation, a large portion of bran protein cannot be extracted by aqueous extraction⁵. Moreover, alkaline extraction procedures, which are normally used to prepare protein concentrates from rice bran, may change its nutritional characteristics, such as conversion of cystine and serine residues of protein to toxic lysinoalanine^{5,6}.

For food purposes rice protein is normally isolated from regular rice flours by enzymatic removal of non-protein components¹¹. Microwave heating is very common in the food industry because of its convenience, rapidity and economy¹². It has been found that microwaves cause different biological effects, depending upon field strength, frequencies, waveforms, modulation, duration of exposures, etc. Physical processing with or without enzyme treatments on protein extraction from heat-stabilized defatted rice bran (HDRB) was evaluated¹³. New value-added product was derived from agricultural by-products : rice bran and soybean meal by means of subcritical water (SW)

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hydrolysis, where effect of temperature (200–220°C), reaction time (10–30 min), raw material-to-water weight ratio (1 : 5 and 2 : 5), was determined on the yields of protein, total amino acids, and reducing sugars in the soluble products¹⁴. Although microwave heating is the main cause of these biological effects, no tangible information either on the causes or the mechanisms of microwave has emerged¹⁵.

In the present study, protein isolates were produced from defatted rice bran meal available in India by various methods like conventional chemical and enzymatic modification as well as by microwave treatment and microwave treatment followed by homogenization.

2 MATERIALS AND METHODS

Materials. Full-fat rice bran was obtained from Sethia Oils Ltd. (Burdwan, W.B., India).

2.1 Preparation of De-oiled bran

The de-oiled bran was prepared by solvent extraction in a soxhlet apparatus using hexane (food grade). After complete extraction the meal was desolventized in a vacuum oven at 60°C and 4 mm Hg for 4 h. The de-oiled meal was stored in desiccator and used for further experiment.

2.2 Sieving of rice bran meal

Rice bran meal was screened to pass through an 80 mesh (1770- μ m openings) sieve. The fraction (through fraction), passing through the screen was used as starting material for further study. Papain (from papaya latex, having activity 100TU/6000NF) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India and Viscozyme 120 L (an enzyme preparation from *Aspergillus* with a wide variety of carbohydrase activities bearing 120 FBG/ml) was a kind gift from Novo Industry A/S Copenhagen, Denmark were used for enzymatic treatment of rice bran.

2.3 Preparation of rice bran protein isolate (RPI)

Deoiled rice bran (through fraction) was mixed thoroughly with 10 times distilled water, pH of the medium was then adjusted to 10.0 and was stirred for 1 h at 50–55°C. The slurry was centrifuged at 4000 $\times$ g for 10 min. The pH of the supernatant liquid was further adjusted to 4.0 with 1N HCl and again stirred for 1 h at 50–55°C, centrifuged at 8000 $\times$ g for 20 min. The solid residue (RPI) thus obtained was collected and dried in a vacuum oven at 50 °C for 10 h.

2.4 Preparation of rice bran protein isolate by microwave treatment

A domestic microwave oven (LG Electronics India Limited, Model MS 257PL, Korea) was used. The experimental setup during microwave heating is shown in Fig 1.

Fig. 1 Experimental setup during microwave treatment.

The slurry containing deoiled bran, after alkaline extraction was adjusted to pH 8.0 and treated in microwave oven for separate time intervals of 20, 30, 40, 60, and 90 sec. All samples of 100 ml volume were simultaneously exposed at a frequency of 2450 MHz and operated at 800 Watt.

2.5 Homogenization of microwave treated meal

After microwave treatment, the slurry was transferred to a homogenizer (REMI Motors, Mumbai, India). The optimum time for homogenization was found to be 10 min. at a speed of 1400 rpm. Then each of the slurry was centrifuged at 4000 $\times$ g for 10 min (Remi Cooling Centrifuge C-24 BL, India). Protein content of both the solid residual meal and supernatant were measured according to Kjeldahl method¹⁶ and Lowry *et al.* method¹⁷ respectively. Each supernatant was then lyophilized and freeze-dried (Freeze Dryer FD-5N, Tokyo Rikakikai Co. Ltd., Japan) and stored at 0°C for further study.

2.6 Preparation of rice bran protein isolate by enzymatic modification

De-oiled rice bran was mixed with water (10% w/v) and pH of the medium was adjusted to 8.0 and treated separately with 0.1% (w/w) papain and 5.0% (v/w) viscozyme for 1 h at 37°C. After enzymatic treatment the mixtures were heated at 95°C for 5 min to inactivate the enzymes. The slurry was centrifuged at 4000 $\times$ g for 10 min. Both the supernatant and solid residual meal was collected separately and their protein content were measured. Each supernatant was then lyophilized and freeze-dried (Freeze Drier FD-5N, Tokyo Rikakikai Co. Ltd., Japan) for further use. The protein isolate obtained by papain treatment is RPIp

and that by viscozyme treatment is RPI_v.

Total nitrogen content was measured according to the Kjeldahl method, which was converted into protein content using a conversion factor of 6.25. Moisture, crude fiber, ash content, nitrogen solubility index (NSI) and protein dispersibility index (PDI) was determined by Official and Tentative Methods of AOCS¹⁶⁾. Silica content was determined by converting about 1 g sample into ash. Then the ash was taken in a platinum crucible and about 10 ml hydrofluoric acid was added and boiled gently in a heater with asbestos gauze. Gradually silica was removed by converting into volatile fluorosilicic acid. After complete volatilization the crucible was strongly heated directly in the heater for 5 min, the crucible was cooled in a desiccator and weighed. The silica content was calculated from the loss of the weight.

3 RESULTS AND DISCUSSION

Commercial rice bran in India is contaminated with rice husk during milling. To reduce the fiber, ash and silica, the rice bran meals were screened through 80-mesh sieve. A fraction (over fraction, yield-30%) rich in fiber, silica and

ash was discarded from the meal. The proximate composition of the de-oiled rice bran meal and its two different fractions (through and over fractions) are shown in Table 1. In the through fraction, the protein content increased from 20.8 to 24.1% and the silica content reduced from 3.1 to 0.4%. Crude fiber and ash content were also decreased from 16.7 to 13.9% and 14.9 to 11.3% respectively by sieving the whole bran meal. The quality of protein in terms of NSI and PDI values were also improved significantly in through fraction. This through fraction with improved quality was used as starting material for further study. Rice bran protein isolates were prepared from this through fraction.

Attempt has been made to increase the protein solubility by physical means like microwave digestion and microwave digestion followed by homogenization. The effect of microwave treatment on the recovery of protein from de-oiled rice bran meal is given in Table 2. Simple 40 s microwave treatment could give the protein recovery of 78.4% as against 36.1% after 1 min of conventional boiling. Further microwave treatment has decreased the protein recovery, probably due to denaturizing of protein. Therefore, 40 s is taken as optimum time for microwave treatment. The

Table 1 Characteristics of deoiled rice bran meal and its two different fractions obtained by sieving.

Characteristics	Deoiled rice bran	Through fraction	Over fraction
Moisture(%)	10.3 ± 0.9	11.0 ± 0.8	8.6 ± 0.4
Protein(%)	20.8 ± 0.8	24.1 ± 1.0	13.1 ± 0.8
Crude fiber(%)	16.7 ± 0.6	13.9 ± 0.8	23.2 ± 0.7
Ash(%)	14.9 ± 0.9	11.3 ± 0.8	21.8 ± 0.8
Silica(%)	3.1 ± 0.3	0.4 ± 0.1	9.3 ± 0.6
NSI	19.6 ± 0.9	29.9 ± 0.7	17.1 ± 0.7
PDI	30.0 ± 0.8	44.0 ± 1.0	29.0 ± 0.8

Values are mean ± s.d., n = 3

Table 2 Percent Recovery of Protein from Deoiled Rice Bran Meal (through fraction) by Microwave Treatment and Microwave Treatment Followed by Homogenization at Different Time Intervals.

Time of microwave treatment (sec)	% Recovery of protein	
	Microwave treatment	Microwave treatment followed by homogenization
20	67.0 ± 0.9	79.9 ± 0.9
30	68.7 ± 0.9	81.6 ± 1.1
40	78.4 ± 1.0	82.6 ± 1.0
60	75.1 ± 1.0	80.4 ± 1.1
90	70.3 ± 1.0	79.2 ± 1.0

Values are mean ± s.d., n = 3

protein recovery was further increased by microwave treatment followed by homogenization as shown in Table 2. A maximum of 82.6% protein has been recovered after 40 s microwave treatment followed by 10 min of homogenization.

Defatted rice bran meal has been treated with enzyme papain and viscozyme separately to increase the protein solubility as well as the yield of protein isolate. Papain is well known for its ability to produce protein hydrolyzates from soybean¹⁸⁾. The authors have already been produced protein hydrolyzates from sesame protein isolate and rice bran protein by using papain^{19, 20)}. The yield of different protein isolates prepared from different treated rice bran meals were shown in Table 3. The yield of protein isolate (RPI), prepared by alkaline extraction followed by acidic precipitation was 10.2%, which was further increased to 14.5, and 22.4% by papain and viscozyme modification respectively. Microwave treatment had increased the yield to 21.1%, which was further increased to 22.3% by microwave treatment followed by homogenization.

Microwave heating is one of the most commonly used methods because of its convenience and economy. The depth of penetration is determined by electrical and physical properties, heating patterns and can vary significantly with chemical composition, product temperature and frequency at which the microwave penetrates. Anderson *et al.*⁷⁾ have increased protein extractability in rice bran by colloid milling followed by homogenization. Here, in the present study protein extractability get increased by simple inexpensive microwave treatment.

The analytical characteristics of different rice bran protein isolates prepared from different treated rice bran meals are given in Table 3. Different undesirable components like crude fiber, ash and silica contents were very low in these protein isolates. Due to very high protein content, these fractions can be utilized in various food and pharmaceutical formulations.

The percentages of protein recovery from different treated meals are compared in Table 4. About 82.5% protein has been recovered as soluble protein from de-

Table 4 Percent Recovery of Protein from Different Treated Deoiled Rice Bran Meal (Through fraction).

Treatment	% Recovery of protein
Alkaline Extraction	36.1 ± 0.9
Papain modification	54.0 ± 1.0
Viscozyme modification	82.6 ± 1.0
Microwave treatment	78.4 ± 0.9
Microwave treatment followed by homogenization	82.5 ± 1.0

Values are mean ± s.d., n = 3

oiled bran by viscozyme treatment and also by microwave treatment followed by homogenization. This suggests that microwave treatment used in this study had disrupted the extensive network of disulfide bonding and aggregation in the proteins. Therefore, microwave treatment along with homogenization is a suitable alternative processes in extracting protein from rice bran meal.

4 CONCLUSION

The maximum of about 83% protein can be recovered as soluble protein from de-oiled bran by 40 sec microwave treatment followed by 10 min of homogenization. Therefore, microwave treatment along with homogenization can be used as a suitable alternative processes in extracting protein from rice bran meal.

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Table 3 Characterization of Different Rice Bran Protein Isolates Obtained from Different Treated Rice Bran Meal.

Treatment	Product	Yield (%)	Protein (%)	Crude fiber (%)	Ash (%)	Silica (%)
a. Alkaline Extraction	RPI	10.2 ± 0.2	86.2 ± 1.1	1.8 ± 0.2	0.3 ± 0.1	0.03
b. Papain modification	RPI _p	14.5 ± 0.1	89.8 ± 1.1	1.8 ± 0.1	0.2 ± 0.1	0.02
c. Viscozyme modification	RPI _v	22.4 ± 0.2	88.9 ± 1.2	1.5 ± 0.1	0.2 ± 0.1	0.02
d. Microwave treatment	RPI _m	21.1 ± 0.2	89.7 ± 1.2	1.4 ± 0.2	0.2 ± 0.1	0.01
e. Microwave treatment followed by homogenization	RPI _{mh}	22.3 ± 0.2	89.4 ± 1.2	1.5 ± 0.1	0.2 ± 0.1	0.01

Values are mean ± s.d., n = 3

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