

Original Research Article

Rice bran Stabilization by Solid-State Fermentation: Effect on Chemical Composition, Functional Properties, and Antioxidant Activity

Abstract

Rice bran is the main by-product of rice grain processing. It is produced in large quantities worldwide and it contains a high amount of valuable nutrients and bioactive compounds with significant health-related properties. Despite that, its application in the food industry is still scarce because of its sensitivity to oxidation processes, instability, and poor technological suitability. Thus, this study aimed to investigate the chemical composition, functional properties, and antioxidant activity of rice bran fermented by lactic acid bacteria (*Lactobacillus plantarum*), and *Saccharomyces cerevisiae* as active dry yeast in the solid state. Bioactive compounds (free, bound, and total phenolic acids), antioxidant activity, water-extractable arabinoxylans and functional properties were evaluated. Similarly, phytic acid degradation was determined in fermented and non-fermented rice bran. After fermentation processes, protein, fiber, and ash contents in modified bran were raised compared to non-modified bran. Total, free phenolic content, and antioxidant activity increased after solid-state fermentation. Phytic acid content decrement of 40.01% and water-extractable arabinoxylans increment four times were observed in fermented bran. As well as, protein solubility, water holding capacity, water-solubility, oil holding capacity of fermented rice bran were enhanced. Thus, the solid-state fermentation is an efficient technique and can be applied to improve the nutritional and functional properties of rice bran, as well as utilized as ingredients in food applications.

Keywords: Rice bran, Lactic acid bacteria, *Saccharomyces cerevisiae*, functional properties, water-extractable arabinoxylans, phytic acid, phenolics, and antioxidant activity

1. Introduction

World rice production in 2020 was approximately 500 million metric tons [1]. Before consumption, almost all rice is milled and as a result, rice bran is

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Like : rice bran, Lactic acid bacteria, functional properties, *Saccharomyces cerevisiae*

obtained in large amounts around 63 million tons annually as a by-product of rice milling [2]. The bran consists of the pericarp, aleurone layer, embryo, and some endosperm, constituting about 10% of the unprocessed rice [3]. Rice bran is rich in protein, essential nutrients like minerals, vitamins, essential fatty acids, dietary fiber, and antioxidants, such as tocopherols and oryzanols [4]. It has been reported that rice bran is mostly comprised of protein (13.2%–18.6%), ash (9.2%–13.9%), lipid (9.5%–22.9%), starch (16.1%–26.7%) and dietary fiber (20.5%–33.3%) [5, 6]. The antioxidants, including anthocyanin and various phenolic acids, were found in rice bran, which has a strong ability to inhibit the formation of or to scavenge the reactive cell-damaging free radicals [7, 8]. Further, the antioxidant capacity of phenolic compounds is associated with health-promoting functions, such as obesity prevention, and anti-cardiovascular disease, as well as anti-inflammatory and anti-cancer effects [9]. In rice grains, phenolic compounds and phenolic acids exist in three forms: soluble free, soluble conjugated, and insoluble bound states [10]. Therapeutic effects were closely related to the nutrient components such as phenolic, dietary fiber, and antioxidants of rice bran [11]. Furthermore, each year 90% of the rice bran produced in the world is utilized cheaply as a feed stock for cattle and poultry and the remainder is used for the extraction of rice bran oil [12, 13], rice bran has very short shelf life owing to high-fat content and potent enzyme lipase which degrades the oil, making bran rancid and inedible.

Phytic acid is an anti-nutritional factor. It is decreasing the dietary bioavailability of nutrients minerals such as Ca^{++} , Mg^{++} , Fe^{++} , and Zn^{++} [14]. In rice kernel, phytic acid is mainly distributed in the outer layers, pericarp and germ [15]. Arabinoxylans are the main components of cell walls in cereal grains. Also, the content of arabinoxylan in rice is highest among all polysaccharides except starch [16], and they exist in both insoluble water and soluble states. The total arabinoxylan content

of rice bran is 4.84%–5.11%, while water-soluble arabinoxylan is 0.35%–0.77% [17]. Numerous investigations have suggested that the insoluble form has negative results on the rheological characteristics of dough [18].

To improve utilization of rice bran, development of feasible stabilization methods and the value-added processes of the nutrients and active compositions are necessary. Many investigations have concentrated on modifying features of rice bran. Numerous investigations have highlighted that fermentation of rice bran with lactic acid bacteria (LAB) or yeast, is a useful pre-treatment process to enhance technological, nutritional, and sensory properties of bran-containing products [11, 19], and to reduce anti-nutritive factors like phytic acid as well as enhancing mineral bioavailability [20]. Similarly, bran fermentation improves bioavailability and the contents of various functional compounds such as total free phenols, and soluble fiber as well as improving functional properties [8, 21]. Therefore, the aim of the current study was to modify autoclaved rice bran by solid-state fermentation with yeast and LAB. Also, study these effects on gross chemical composition, functional properties, phenolics, phytic acid contents, and antioxidant activity of rice bran.

2. Material and Methods

2.1 Raw materials

Commercial rice bran was obtained from Kafr El-Sheikh Mills company (Kafr El-Sheikh, Egypt), *Saccharomyces cerevisiae* active dry yeast (Commercial baker's yeast with high sugar tolerance) was obtained from local market, and *Lactobacillus plantarum* ATCC 14917 was purchased from Microbiological Resources center (Cairo MIRCEN) Ain Shams University, Cairo, Egypt.

2.2 Inoculant preparation

Lactobacillus bacteria strains (*Lactobacillus plantarum*) were activated by inoculating in sterile MRS broth (9 ml) and incubation at 37 °C for 24 h. The cells were separated from the broth by centrifuging and re-suspended in sterile saline solution (9 ml) with final concentration 10⁸ CFU/mL[22]. Active dry yeast was used directly without incubation.

2.3. Solid-state fermentation

The fermentation processes of autoclaved Rice bran (121°C at 15 min), was carried out as follows:

Group □ treated by *L. plantarum*: The autoclaved water (2:1) was added to rice bran, the activated culture of probiotic bacteria (10⁸ CFU/mL) was added to autoclaved bran by a concentration of 1% and incubated at 37 °C for 24 and 48h.

Group □ treated by yeast: The autoclaved water (2:1) was added to rice bran and 1.25% active dry yeast was mixed well and fermented for 24 and 48h.

Group □ treated by LPY: The autoclaved water (2:1) was added to rice bran, the activated culture of probiotic bacteria (10⁸ CFU/mL) was added to autoclaved bran by a concentration of 1% and 1.25% active dry yeast was mixed well and fermented for 24 h and 48h. Rice bran autoclaved and non-autoclaved samples without starters were incubated at 37 °C and used as control. After fermentation all samples were air-dried, then milled by a laboratory mill (Braun, Germany) to pass through a 60-mesh sieve and stored at -20°C until analyses.

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2.4. Gross chemical composition of Rice bran:

The chemical composition of fermented and non-fermented rice brans including protein, fat, fiber, and ash contents were determined according to official methods as described in [23] Carbohydrate was calculated by difference.

2.5 Phytic acid:

The phytic acid was determined in terms of its phosphorous content, using the method described by [24].

2.6 phenolic compounds:

Total phenolic content was extracted from rice bran samples using methods described by the method of [25], while free phenolic compounds were extracted according to [26]. Total and free phenolic content determined by the Folin–Ciocalteu’s method according to [27]. The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 gram of bran sample on dry weight basis. Bound phenolic compounds were calculated by subtract free phenolics from total phenolics.

2.7 Determination of antioxidant activity

Samples were extracted using methods described by [28]. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was carried out according to the method described by [29] with some modifications. The stock reagent solution (10^{-3} Mol) was prepared by dissolving 22 mg of (DPPH) in 50 ml of methanol and stored at 20°C until use. The working solution (6×10^{-5} Mol) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer (6505 UV/Vis, Jenway LTD., Felsted, Dunmow, UK). Extract solution of tested samples (0.1 ml) were vortexed for 30 s with 3.9 ml of DPPH solution and left to react for 30 min, after which the absorbance was measured at 515 nm and recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

DPPH radical scavenging activity (%) = $[(Ab \text{ control} - Ab \text{ sample}) / Ab \text{ control}] \times 100$

Where Ab is the absorbance at 515 nm.

2.8 Water extractable arabinoxylans (WEAX)

The WEAX was determined following previous study [30] by extracting 0.4 g of bran samples with 20 mL of distilled water at room temperature. The extracts were centrifuged at 5000 rpm for 10 min. 100µL of supernatant, 100µL of distilled water and 2 mL of freshly prepared reaction solution (1 g phloroglucinol in 5 mL anhydrous ethanol, 2 mL chlorohydric acid, 110 mL acetic acid, 1 mL 17.5 g/L glucose solution) were pipetted into stoppered glass tube. The tubes were vigorously boiling in water bath for 25 min and then cooled in flowing water immediately. The absorbance was measured at 552 nm and 510 nm successively. D- (+)-Xylose was used as standard. Calculate the content of WEAX by subtraction of the absorbance at 510 nm from 552 nm and comparison of the results with a standard curve and conversion of D-(+)-xylose to pentosan with a scaling factor (0.88).

2.9 pH value:

The pH of the samples was determined according to approved methods 02-52 and 02-31 [31]. one gram of fermented and non-fermented Rice brans was first diluted and homogenize in 10 ml of distilled water then the pH of suspension was measured by a pH meter (Hanna Instruments, Padova, Italy).

2.10. Functional properties

Water-holding capacity and water solubility was achieved according to the method of [32]. Oil Holding Capacity was determined according to the method described by [33]. The protein solubility was achieved according to the method of [34].

2.11 Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) using SPSS software (IBM SPSS Statistics, version 22). When the difference between

Table 1. Gross chemical composition of fermented and non-fermented rice brans (D.W bases)

the samples was statistically significant ($p < 0.05$), the Duncan test was used to determine the differences among the mean.

3. Results and Discussion

3.1 Chemical composition

The results of gross chemical composition of fermented and non-fermented rice bran were illustrated in Table 1. It could be observed that the rice bran raw (RBR) contained 17.81% protein, 8.74% crude fiber, 6.99% ash, 17.27% fat, and 49.19% carbohydrate. The gross chemical composition of RBR in this work are agreement with that reported by [5, 6]. The protein, crude fiber and fat of rice bran was increased after treatment by fermentation compared to autoclaved sample RBA, the high protein content recorded for sample RBYP48h (21.13 %) followed by sample RBP48h (19.56 %) and RBP24h (19.44%). It's been reported that, yeasts and lactic acid bacteria have some proteinases and peptidases [35], while crude fiber increased from 7.02% to 8.64% in RBA and RBY48h samples, respectively, similar results were reported by [36]. In the other hand, there are slight differences in ash content of rice bran after fermentation process compared to non-treated sample (RBR). Small decreased differences were observed in carbohydrate contents of modified rice bran comparison with unmodified sample.

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Samples	protein	fiber	Ash	fat	Carbohydrate*
RBR	17.81±1.20c	8.74±0.93a	6.99±0.19bc	17.27±0.73c	49.19±5.69b
RBA	16.98±1.09d	7.02±0.19c	6.86±0.86 ^{bcd}	17.16±0.84c	51.98±3.44a
RBYP24h	18.40±0.60c	8.43±0.42ab	7.13±0.03b	17.80±0.13 ^{bc}	48.24±0.92bc
RBYP48h	18.48±0.52c	8.64±0.32a	7.68±0.35a	18.10±0.10 ^{abc}	47.10±0.65c
RBP24h	19.44±0.46b	7.47±0.21c	6.54±0.05d	18.13±0.37 ^{abc}	48.43±5.09bc
RBP48h	19.56±0.44b	8.29±0.12ab	6.69±0.14cd	18.47±0.53ab	46.99±5.52c
RBYP24h	19.35±0.35b	7.74±0.23bc	6.67±0.27cd	18.87±0.27a	47.37±4.78c
RBYP48h	21.13±0.42a	8.45±0.17ab	7.14±0.03b	18.97±0.77a	44.31±5.74d

*Carbohydrate by deference

Values are the mean of triplicate determinations with standard division.

The different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

RBR raw bran; **RBA** autoclaved rice bran; **RBYP24h** fermented autoclaved bran with yeast for 24h; **RBYP48h** fermented autoclaved bran with yeast for 48h; **RBP24h** fermented autoclaved bran with lactic acid bacteria for 24h; **RBP48h** fermented autoclaved bran with lactic acid bacteria for 48h; **RBYP24h** fermented autoclaved bran with lactic acid bacteria and yeast for 24h; **RBYP48h** fermented autoclaved bran with lactic acid bacteria and yeast for 48h

3.2. Phenolic compounds and antioxidant activity of fermented and non-fermented rice bran.

The effect of fermentation on phenolic compounds and antioxidant activity of rice bran are presented in Table 2. The results indicated that the free phenolic, total phenolic content and antioxidant activity of rice bran under different fermentation conditions by yeast, LAB, and Yeast combined with LAB were increased after fermentation compared to untreated sample. The highest free content of modified rice bran was observed in RBP48h (721.03 mg GAE /100g), RBYP48h (672.39 mg GAE/100g), and RBYP48h (665.41 mg GAE /100g), while the lowest 586.34 mg GAE /100g was for RBA. While the high of total phenolic content was recorded as follows: 852.33 mg GAE/100g > 806.64 mg GAE/100g > 781.83 mg GAE/100g > for RBYP48h, RBP48h, RBYP24h, respectively. The increase in the total and free phenolic content can be explained by the ability of yeast and LAB to degrade

organic materials due to their highly efficient enzymatic system. Similar results have been previously reported by [37, 38].

From the same table, it was observed that arabinoxylans after fermentation, the content of water-extractable arabinoxylans in rice bran improved significantly ($P < 0.05$) Table 2, increasing from 2.75 (WBR) to 4.72 mg/g for (WBY48h) sample. This enhancement means that fermentation, could be caused the transition from water unextractable arabinoxylans to water-extractable arabinoxylans partly. Water extractable arabinoxylans in bran increased after fermentation, and the highest content was observed in RBPY48h (4.72 mg/g), RBP48h (3.16 mg/g), RBY48h (3.07 mg/g), and RBPY24h (2.91 mg/g), while the lowest 2.12 mg/g was for RBP24h, Similar results have been previously reported by [39, 40].

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Table 2. Phenolic compounds, antioxidant activity and water extractable arabinoxylans of fermented and non-fermented rice bran

Samples	Total phenolic content mg/100g			Antioxidant activity (%)	Water extractable arabinoxylans mg/g
	Total	Bound	Free		
RBR	771.22±1.20c	115.22±0.63b	656.00±1.00d	17.77±0.17g	1.13±0.03g
RBA	691.58±2.83d	105.24±0.92b	586.34±0.66g	19.73±0.70cd	1.18±0.01g
RBY24h	762.31±6.69c	158.21±6.60a	604.10±5.14e	20.56±0.56b	2.75±0.02e
RBY48h	753.38±4.62c	80.99±8.00b	672.39±3.39b	21.75±0.05a	3.07±0.02c
RBP24h	770.13±9.87bc	172.80±7.90a	597.33±3.17f	18.89±0.09ef	2.12±0.03f
RBP48h	806.64±5.36b	85.61±1.38b	721.03±4.03a	20.26±0.07bc	3.16±0.09b
RBYP24h	781.83±7.04bc	188.96±3.40a	592.87±2.76fg	18.43±0.06f	2.91±0.05d
RBYP48h	852.33±3.67a	186.92±2.10a	665.41±3.59c	19.41±0.41de	4.72±0.06a

Values are the mean of triplicate determinations with standard division.

The different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

RBR raw bran; **RBA** autoclaved rice bran; **RBY24h** fermented autoclaved bran with yeast for 24h; **RBY48h** fermented autoclaved bran with yeast for 48h; **RBP24h** fermented autoclaved bran with lactic acid bacteria for 24h; **RBP48h** fermented autoclaved bran with lactic acid bacteria for 48h; **RBYP24h** fermented autoclaved bran with lactic acid bacteria and yeast for 24h; **RBYP48h** fermented autoclaved bran with lactic acid bacteria and yeast for 48h

3.3. Phytate phosphorus, phytic acid degradation, phytic acid and pH values of fermented and non-fermented rice bran

Table 3 shows the effect of fermentation on phytate phosphorus, phytic acid degradation, phytate content, and pH values of rice bran. It was observed that the highest values of phytate phosphorus (0.85%) and phytate content (3.02%) was recorded in raw rice bran. In contrast, the phytate phosphorus of fermented rice bran decreased significantly ($P < 0.05$) from 0.75 to 0.51%, while phytate content decreased from 2.66 to 1.81%, and the highest phytate phosphorus 0.75% and phytic acid 2.66% was documented in RBY24h, followed by RBP24h (0.73 and 2.59%), RBY48h (0.66 and 2.34%), and RBYP24h (0.62 and 2.20%), the lowest 0.51% and 1.81% were recorded in RBP48h, these result in same line reported by [20]. Fermentation has been shown to reduce the phytic acid content of grains because of the action of phytases produced by yeast or lactic acid bacteria [41]. In the same trend, the high value of phytic acid degradation were recorded as follows: 40.01% > 37.75% > 27.15 > 22.52% for RBP48h, RBYP48h, RBYP24h, and RBY48h respectively, whereas phytic acid degradation was zero in RBC sample (Table 3). Phytic acid degradation may be due to phytase, and phosphatase enzymes present in microorganisms which can hydrolyze the phytic acid expressed in *Lactobacillus rhamnosus* 1473, as previously reported in strains of the same species [42, 43]. The pH reduction caused by the production of carbon dioxide and organic acids by yeast increases phytase activity thereby, increasing phytic acid solubility. Results regarding the pH values, was observed that a considerable decrease in pH values was (from 6.51 ± 0.01 to 5.15 ± 0.06).

Table 3. Phytate phosphorus, phytic acid degradation, phytic acid and pH values of fermented and non-fermented rice bran

Samples	Phytate phosphorus %	Phytic acid degradation %	Phytic acid %	PH
RBR	0.85±0.12 ^a	00	3.02±0.44 ^a	6.51±0.01 ^a
RBA	0.81±0.25 ^{ab}	4.97	2.87±0.09 ^{ab}	6.50±0.08 ^a
RBYP24h	0.75±0.01 ^b	11.92	2.66±0.21 ^b	6.20±0.04 ^b
RBYP48h	0.66±0.06 ^{cd}	22.52	2.34±0.16 ^{cd}	5.78±0.05 ^e
RBP24h	0.73±0.04 ^{bc}	14.24	2.59±0.31 ^{bc}	6.11±0.03 ^c
RBP48h	0.51±0.01 ^e	40.01	1.81±0.37 ^e	5.15±0.06 ^g
RBYP24h	0.62±0.08 ^d	27.15	2.20±0.19 ^d	5.98±0.07 ^d
RBYP48h	0.53±0.09 ^e	37.75	1.88±0.33 ^e	5.54±0.10 ^f

Values are the mean of triplicate determinations with standard division.

The different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences

RBR raw bran; **RBA** autoclaved ricebran; **RBYP24h** fermented autoclaved bran with yeast for 24h; **RBYP48h** fermented autoclaved bran with yeast for 48h; **RBP24h** fermented autoclaved bran with lactic acid bacteria for 24h; **RBP48h** fermented autoclaved bran with lactic acid bacteria for 48h; **RBYP24h** fermented autoclaved bran with lactic acid bacteria and yeast for 24h; **RBYP48h** fermented autoclaved bran with lactic acid bacteria and yeast for 48h

3.4. Functional properties of fermented and non-fermented rice bran.

The results in Table 4 indicate the functional properties of fermented and non-fermented rice bran. It was observed that the functional properties of rice bran including the water holding capacity, water-solubility, oil holding capacity, and protein solubility of fermented rice bran were improved and showed higher values than that observed for non-fermented sample and showed significant variations at ($p \leq 0.05$). The highest WHC was recorded in RBYP48h (223.26%), followed by RBP48h (219.12%), RBYP48h (218.95%) and RBYP24h (218.61%). while the lowest was in RBR (213.33%), Similar results have been previously reported by [44]. These results demonstrate that all fermentation can be improving the WHC of rice bran. This increase in WHC may be due to an increment of soluble dietary fiber because it can absorb more water than insoluble dietary fiber. It has been reported that fermentation by lactic acid bacteria or yeast can enhance the content of soluble

dietary fiber of bran[45, 46].In addition, the water solubility (WS)of row rice bran was12.14%, and it was significantly increased up to 17.50% after fermentation (p <0.05). This increase in WS of modified rice bran could be due to the increase of soluble dietary fiber.

After-fermentation the OHC of rice bran was enhanced obviously. The mean values showed higher oil holding capacity for RBYP48h (187.76%), followed by RBP24h (184.10%), RBPY24h (183.59%) and RBY48h (183.45%), while the lowest 176.50% was found in RBA. Similar results of wheat bran have been previously reported by[46].Regarding protein solubility results (Table 4),the protein solubility of raw rice bran (RBR) was 10.85%, while autoclaving and fermentation processes increased the soluble protein of rice bran significantly (p <0.05). The highest values were recorded as follows: 16.04% > 14.93% > 14.13% > 13.83% for RBYP48h, RBP48h, RBY48h, and RBYP24h, respectively. These results are in line with those reported by[47].These increasing in soluble protein, maybe due to degradation of proteins in rice brans after fermentation process of rice bran treatment by yeast or lactic acid bacteria. It was reported that Lactic fermentation enhanced indirectly protein hydrolysis via endogenous proteases[48].

Table 4. Functional properties of and non-fermented rice brans (D.W bases)

Samples	Water holding capacity (%)	water solubility	Oil holding capacity (%)	soluble protein as % of total sample protein
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RBR	213.33±1.67 ^c	12.14±0.36 ^d	176.88±1.88 ^d	10.85±0.50 ^e
RBA	215.18±1.82 ^d	11.89±0.53 ^d	176.50±1.55 ^d	12.54±0.40 ^d
RBYP24h	217.83±0.45 ^{bc}	12.66±0.33 ^d	182.33±1.67 ^c	13.74±0.87 ^c
RBYP48h	218.95±1.63 ^b	13.98±0.02 ^c	183.45±0.96 ^b	14.13±0.26 ^c
RBP24h	216.87±2.04 ^c	14.23±0.77 ^{bc}	181.37±1.41 ^c	12.85±0.87 ^d
RBP48h	219.12±0.97 ^b	15.00±0.50 ^b	184.10±1.80 ^b	14.93±0.07 ^b
RBYP24h	218.61±1.47 ^b	14.11±0.65 ^c	183.59±1.24 ^b	13.83±0.17 ^c
RBYP48h	223.26±0.75 ^a	17.50±0.47 ^a	187.76±2.10 ^a	16.04±0.46 ^a

Values are the mean of triplicate determinations with standard deviation.

The different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

RBR raw bran; **RBA** autoclaved rice bran; **RBYP24h** fermented autoclaved bran with yeast for 24h; **RBYP48h** fermented autoclaved bran with yeast for 48h; **RBP24h** fermented autoclaved bran with lactic acid bacteria for 24h; **RBP48h** fermented autoclaved bran with lactic acid bacteria for 48h; **RBYP24h** fermented autoclaved bran with lactic acid bacteria and yeast for 24h; **RBYP48h** fermented autoclaved bran with lactic acid bacteria and yeast for 48h

4. Conclusion

Our data demonstrated that solid-state fermentation is an efficient technique for improving rice bran's nutritional and functional characteristics. After fermentation, protein, fiber, ash contents as well as phenolic compounds, and antioxidant activity increased, phytic acid content reduced partly, while water-extractable arabinoxylans and protein solubility increased significantly ($p < 0.05$). In addition, the functional properties of fermented rice bran including water holding capacity, water-solubility, and oil holding capacity were improved. Solid-state fermentation enhances the bioavailability of rice bran as well as increases its use as a functional ingredient in the food industry.

5 -References

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