



Optimization of the conditions for rice bran phytate degradation by their own phytases

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ABSTRACT

The rice bran by-product of rice during milling process chemically contains moisture, ash, crude protein (12%), crude fat (15%), crude fiber 7%, crude carbohydrate (31.1%), and energy. It contains high phytate and dietary fiber contents. The cereal whole paddy after milling produces 8% of rice bran. Rice bran amino acid profile has been normally suggested to be superior to cereal grain proteins. Existing phytase within the rice bran generally improves phytate phosphorus utilizations. The present study has been conducted to find the effect of phytase activity of rice bran against five different temperatures and also the effect of phytase activity of rice bran against five different temperatures at optimum pH 5.5. The results showed that as the concentration of rice bran increases from 0 to 20%, the phytase activity also increased. The optimum phytase activity was found at 10% concentration of rice bran at 37°C. Moreover, an increase in phytase activity at the same concentration of rice bran was observed while adjusting the pH at pH 5.5. The four concentrations of rice bran showed gradual increase in activity at 50°C which was directly proportional to the concentrations of rice bran and comparatively better while adjusting the pH at pH 5.5. Around two-fold increase in activity was observed at room temperature and 50°C when the concentration of rice bran was increased from 5 to 20% with and without pH 5.5 adjustments.

1. INTRODUCTION

Rice is produced around 670 MMT every year compared to 680 MMT for wheat, 440 MMT for oilseeds, and 1090 MMT for coarse grains in 28% world's landscape. 161.1 million hectares of rice-cultivated area throughout worldwide [1] and around 114 nations grow rice and more than 50 have yearly productions of 100,000 metric tons or more. There are more than 100 varieties of rice are grown in the world accounting for about 25% of the world's nourishment grain generation [2]. China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, Japan, and the United States are the 11 countries producing 28.2%, 22.4%, 8.8%, 6.5%, 5.9%, 4.6%, 4.2%, 2.4%, 1.7%, 17%, and 13% of the world production of rice. The major paddy producers China and India with their pooled production account nearly half of the total world's paddy production. Milling of paddy yields 70%

of rice (endosperm) as the major product and byproducts comprising 20% rice husk, 8% rice bran, and 2% rice germ [3,4]. The world's 70% population has the principal food as rice and its one-tenth of the harvested rice is rice bran (approximately 60 million metric tons). In 2001, The world production of paddy in 2017 was 711.5 MMT [5]. Consistently, every year huge amounts of rice bran around 63 to 76 million tons produced in the world and over 90% rice bran are sold as animal feed. Diverse food products can be formulated with 5–10% rice bran [6-8].

The nutrient content of rice depends on assortment of rice soil and on the conditions they develop. Pre-germinated brown rice has protein 14.6 g/100 g than white rice 7.3 g/100 g. Whereas, the fat content is higher 24.8 g/100 g for pre-germinated brown rice than white rice 1.5 g/100 g [9]. Commercial rice types such as brown, parboiled brown, parboiled milled, and milled rice had similar protein and crude fat contents [10]. Different rice growing conditions in various regions of the earth widely influence the type of paddy growth. The main factors such as (i) situation of the land, (ii) soil type, (iii) rice variety, (iv) climatic conditions, (v) degree of rainfall, (vi) irrigation methods and resources, and (vii) special agronomic features.

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The rice bran which is the side effect of rice during milling process chemically contains moisture, ash, crude protein, crude fat, unrefined fiber, unrefined starch, and energy. Rice bran especially composed of protein, fiber, oil, vitamins, minerals, and starch which come from endosperm in the course of polishing [11]. In Japan, rice bran is the substrate of gamma-aminobutyric acid which reduces the risk of hypertension or neural dysfunction [12]. Defatted rice bran contains various polysaccharide and dietary fiber that helps in cancer and cardiovascular diet regimen treatment [13,14]. The nutrients and phytochemicals in bran help the plant, and for human, the bran provides energy and some protein, antioxidants, and B vitamins [15]. Rice bran gives the majority of daily calories for many companion animals and humans [16].

The total dietary fiber content in parboiled rice and stabilized rice bran (SRB) is 27.0% and 20.9%. Rice bran consists of crude protein 12%, fat 15%, fiber 7%, and carbohydrate 31.1%. It additionally contains 0.3 mg/g Ca, 5 mg/g Mg, 9 mg/g phytin, 43 µg/g Zn, 12 µg/g thiamine, 1.8 µg/g riboflavin, and 267 µg/g niacin. The high phosphorous content is about 11 mg/g and silica is about 6 mg/g [17]. It incorporates varying measures of scraps of husk and impurities.

The rice bran is a pericarp or outer cuticle layer that remains underneath the hull. It gets expelled amid the processing procedure. The rice bran was scorched before a long time since considered as waste. Recent studies proved its nutritive significances. The rice bran derived from different varieties of rice contains antioxidant compounds like polyphenols, carotenoids, vitamin-E and tocotrienol which help in preventing the damage of body tissue [18]. The rice bran has 18–20% of fat, 14–15% protein, and apparent measures of minerals and vitamins. The rice bran and the bran oil have market values now.

Regular uses of rice bran are in snack foods, bakery products, dough conditioners, beverages, gluten-free foods and medical foods, isotonic drinks, iced tea drinks, enhanced juices, and mineral supplements. “Rice milk” non-dairy option to milk is produced using naturally developed rice.

Rice bran could be incorporated up to 10% without any adverse impact on laying performance, egg quality, and digestive organs [19]. Commercially by adding rice bran in the feed diets will have much profitable economic prospective.

Of 100 kg of paddy on milling yields, 56–58 kg white rice, 10–12 kg broken rice, 18–20 kg husk, and 10–12 kg rice bran. The lipase in rice bran rapidly degrades the oil making the bran foul and unpalatable. Stabilized (by heating 135°C for 3 s at 15% moisture and holding the extruded bran at 99°C for 3 min preceding cooling for deactivating the lipase) rice bran (SRB) has the stability around half year and could possibly be utilized as a food ingredient and additionally as a horse feed supplement. The wholesale price for SRB ranges from \$2.92 to \$4.25/kg, whereas rice bran and rice bran de oiled price range from \$2.92 and 4.25/kg.

The partial replacement of maize and wheat with processed rice bran increased the body weight gain of chicks [20]. The rice bran did not modify development rate, nourish admission, or feed conversion ratio (FCR) (feed transformation proportions) of duckling at 17th day of age. The full-fat rice bran can be used up to 45% [21] and when the rice bran level of 60% and 74.7% was used in feeds, it affected the egg production, shell thickness, and yolk color [22,23]. These days the rice brans are acquired by processing naturally developed excellent parboiled rice with high-protein content are generally utilized as a

cattle and poultry feed. In case of chicken diets, broilers and hens (0–8 weeks) up to 10%; hens (8–20 weeks) up to 20%; and laying hens up to 30% rice bran quantity are recommended [24].

The protein (12–16%) and fiber (10–28%) content of rice bran vary depending on the manufacture and process. The rancidity in meat was prevented by phytic acid (PA) without risking health [25]. PA level in the different rice constituents in the germ with 7.6 g 100 g⁻¹ and endosperm with 1.2 g 100 g⁻¹ [26]. PA ranges from 5.94 to 6.09 g 100 g⁻¹ [27] and responsible for antioxidant capacity and potential beneficial effects on health. Significant measure of minerals and vitamin was found in the rice bran of BRRRI-28 [28]. Rice bran available as meal and pellet forms in 50 kg plastic bags, rice bran or cattle, fish, and animal feed cost the US \$ 500–600/Metric Ton.

Phytase can improve the nutritive value of rice bran. The growth rate increased by the addition of phytase in duck diet containing 20% rice bran [18,20]. Phytase hydrolyzes the phytate molecule, discharging P and in the meantime decreasing the concentration and in this way the counter nutritious impacts of phytate [29]. Phytase (500 unit/kg) addition to broilers diets based on corn, wheat, or barely increased apparent metabolizable energy in corn diets [30]. The FCR is generally increased (i.e. become poorer) as rice bran in that diet formula increased. The rice bran (35%) with microbial phytase (1050 or 1400 I.U phytase/kg diet) enhanced the hens day egg creation of layers (86.7% and 88.2%, individually) to be equivalent to that the production (88.9%) of birds on the control (0% rice bran) diet. The eggshell thickness was found increased whereas ineffective in influencing the yolk color.

More elevated amount of rice grain decreases expensive feed price than corn or soya or corn soya-based feeds. Its few sorts of lipases drive increment in free unsaturated fats (FFA) by hydrolyzing the oil. Fast increment in the free unsaturated fat occurs up to 7–8% within 24 h [31]. The enzyme was dynamic up to 40°C and the activity declined pointedly to 65% at 60°C and afterward gradually decreased [32]. The hydrolytic rancidity extremely influences the nutritive value and tastefulness of rice bran [33]. Rice bran contains 15–22% oil by weight [34,35]. The common rice bran is a light tan, unstable meal containing a variety of particle sizes. The deleterious effects were overcome by autoclaving the bran [36].

The rice bran has no any toxicity detected toward animal and human. Furthermore, it is not harmful for the end product. Higher in fiber, bulky, and nutritional valued rice bran becomes rancid due to its high unsaturated fat content. However, still the rice bran is an ideal choice. Higher intake of rice bran as a whole grain was connected with a brought down danger of hypertension [37]. The dietary rice bran may exert valuable impacts against breast, lung, liver, and colorectal cancer [14]. Therefore, in our present study tried to use the constitutive rice bran phytases for dephosphorylation and degradation of rice bran to increase the nutritional value of rice bran to optimize by selecting optimal rice bran concentration, temperature, and pH for enzyme dephosphorylation.

2. MATERIALS AND METHODS

2.1. Raw Material and Chemicals

The rice bran (after milling of rough rice of *Oryza sativa* L. CV) was kindly given by Ministry of Primary Resources and Tourism, Brunei Darussalam. The bran size 250µm screen (Mesh no. 60).

The chemicals used were 5 N sulfuric acid: 10 mM ammonium molybdate, 1.0 M citric acid, and sodium acetate buffer were purchased

from Sigma-Aldrich Chemical Co., (St. Louis, Mo, USA). High-performance liquid chromatography grade ethyl acetate and ethanol were purchased from BHD (Poole, UK) and were obtained from Fluka Chemical (Buchs, Switzerland). All chemicals and reagents were an analytical grade.

2.2. Effect of Phytase Activity of Rice Bran at Various Temperatures

The rice bran with different concentrations from 0 to 20% (w/v) in sterilized distilled water was prepared. All the mixture were stirred for 10 min and were incubated in the sterilized 20 ml glass bottles at five various temperatures (4°C, room temperature [RT 25°C], -18°C, 37°C, and 50°C) for 1 h. After incubation, these slurries were centrifuged for 10 min at 10,000 rpm. The supernatants were decanted and measured for phytase activity in a MR96A microplate reader.

2.3. Phytase Activity Assay

The supernatant of rice bran was subjected to phytase measurements at 28°C. Exactly 50 µl of supernatants were used. The liberated inorganic phosphate was measured utilizing an alteration of the ammonium molybdate strategy [38]. A newly prepared solution of acetone: 5 N sulfuric acid: 10 mM ammonium molybdate (2:1:1 v/v) and thereafter 10 µl of 1.0 M citric acid were added to 50 µl of the rice bran mixture for the estimation of absorbance. The phytase activity with and without rice bran (control) was carried out. The rice bran filtrates were measured for phytase activity in microplate reader MR96A (Shenzhen Mindray BioMedical Electronics Co., Ltd.) at a primary wavelength of 405 nm and a secondary wavelength of 450 nm. To quantify the phosphate released, a calibration curve was constructed within the range of 5–1200 mM phosphate.

The phytase activity was calculated from an average of at least three phytase assay measurements. One unit (U) of phytase activity was defined as the concentration of inorganic phosphate, in µmol, released per min per mL of enzyme preparation (U/ml). The activity was calculated using the slope of the straight line resulting from the assay with the sample (absorbance/minute) and the slope of the straight line arising from the standard curve (absorbance/µmol of P).

2.4. Effect of Phytase Activity of Rice Bran Against Five Different Temperatures at Optimum pH 5.5

The rice bran with different concentrations of 0–20% (w/v) and pH was adjusted to 5.5 using sterilized 1 mM sodium acetate buffer solution. The mixture was after stirring for 10 min. They were incubated in sterilized 20 ml glass bottles at five various temperatures (4°C, RT [25°C], -18°C, 37°C, and 50°C) for 1 h. After incubation, these slurries were centrifuged for 10 min at 10,000 rpm and the supernatants were decanted. The phytase activity was measured in a MR96A microplate reader as before [23].

3. RESULTS AND DISCUSSION

The phytase activity of rice bran without any additives tested at various temperatures of incubation showed diverse results. The increase in phytase activity in all concentrations of rice bran was observed, and the activity increased from RT to 50°C when the rice bran concentrations increased from 0 to 20%. The rice bran concentration (10%) at 37°C showed optimum activity. Even at -20°C and 4°C considerable activity was noticed. The phytase activity varied regardless of temperature at fixed rice bran concentrations. Higher activity was found in 20% rice bran concentrations than 5% [Figure 1].

The phytase activity of rice bran without any additives was tested at different incubation temperature with adjusted pH 5.5 also showed varied results. The phytase activity was clearly noticed in all various concentrations. Increase in activity noticed when rice bran concentrations increased at all temperatures. Rice bran concentration (10%) at 50°C showed optimum activity at pH 5.5. Even at -20°C and 4°C substantial activity was noticed. The lipase was found active up to 40°C and its activity declined at 60°C was reported earlier [32], by changing the pH of rice bran, the optimum phytase activity was obtained at 50°C.

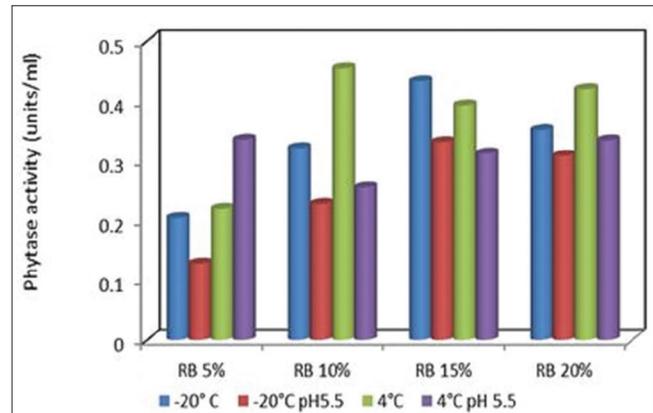


Figure 1: Phytase activity (units/ml) Vs various concentrations of rice bran with and without pH 5.5 at -20°C and 4°C

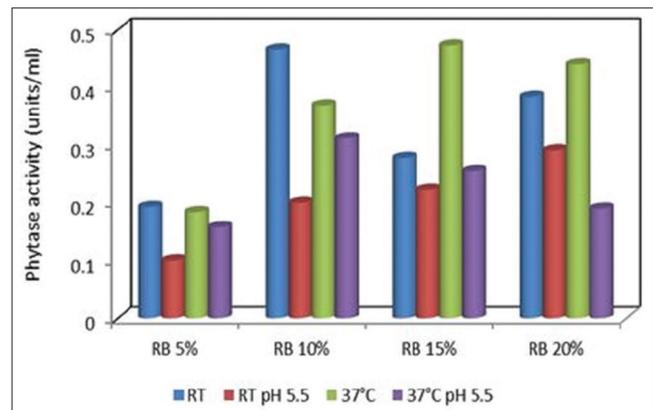


Figure 2: Phytase activity (units/ml) Vs various concentrations of rice bran with and without pH 5.5 at RT (25°C) & 37°C

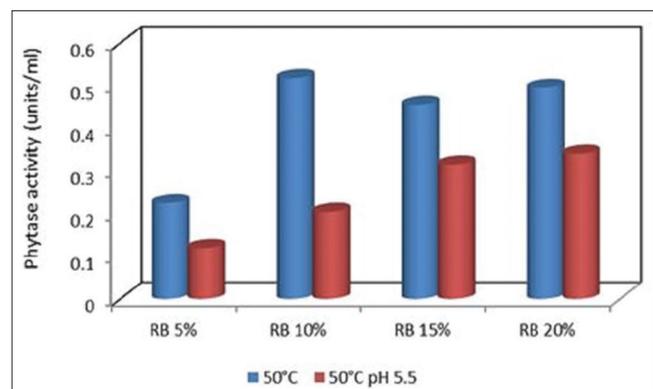


Figure 3: Phytase activity (units/ml) Vs various concentrations of rice bran with and without pH 5.5 at 50°C

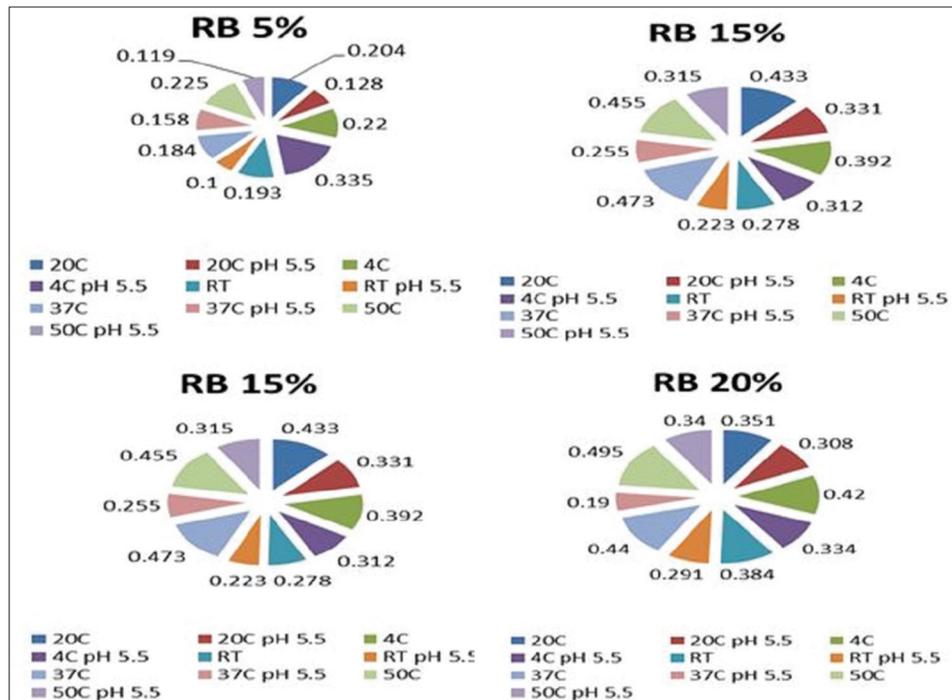


Figure 4: Comparative study of Phytase activity of different concentrations of rice bran without additives at various temperatures

In general, the pH of commercial rice bran varies from 6.0 to 6.9. The enzyme presents in rice bran is temperature and pH sensitive. The rice bran lipase is known to have a pH optimum of 7.5–8.0 at 37°C. Furthermore, activity reduced depends on the increase or decrease in pH levels. The enzyme was inactivated by incubation for 15 min at 60°C was reported earlier [39]. Approximately 90% of rice bran phosphorous is a product of phytate degradation. In the pH range 4.1–5.2 (acidic), the solubility of total phosphorous is maximum of 80% and increased at 5 to pH 6.2. At pH 2 (highly acidic), the total phosphorous solubility was very low was also reported [40]. The rice bran enzyme glutamate decarboxylase was active at pH 5.5 and 40°C and retained 80% of its original activity in the pH range 5–9 and the temperature range 30–50°C was also reported [41]. The enzyme activity for phytase degrading enzymes from rice bran has pH 5.5 as optimal for plant enzymes. The cereal phytases have optimal pH of 5.0–6.0. The phytase from rye with optimal activity for dephosphorylation at pH 6 was also reported [42,43]. At same concentration of rice bran at constant optimum pH, varied phytase activities irrespective of temperature was also observed. Higher activity was found in 20% concentrations than 5% at all different temperatures [Figure 2].

The existing phytase within the rice bran improves phytate phosphorus utilization [29] can be a reason for general phytase activity increase with the increase in rice bran concentrations. Addition of phytase to diets containing rice bran definitely improved further [30]. Furthermore, by autoclaving the rice bran, deleterious effects can be overcome may need further study. In case of chicken diets, broilers and hens (0–8 weeks) up to 10%; hens (8–20 weeks) up to 20% rice bran, and in duck diet containing 20% rice bran were reported [44]. When comparing the rice bran at pH 5.5, the results showed that the phytase activity increased than the rice bran without pH adjustment. The phytase activity corresponding to the gradual increase in rice bran concentrations is shown in Figures 1-3. A comparative study of phytase activity of various concentrations of rice bran without

additives at various temperatures is individually shown in the pie charts in Figure 4.

4. CONCLUSION

The data presented showed optimal activity of phytase was found at the concentration of rice bran 10%, at 37°C, and pH of 5.5. The time of incubation, temperature optima, and rice bran concentrations were also important for efficient dephosphorylation processes. The study concludes rice bran phytase was still active after milling and polishing processes of rice.

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