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Research Article

# A New Low-protein Foodstuff from Processed Brown Rice for Chronic Kidney Disease

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## Abstract

**Aims:** Chronic kidney disease (CKD) is increasing in prevalence worldwide, not only as a complication of diabetes and end-stage glomerulonephritis but also as a typical degenerative process in the elderly. A low-protein diet is essential for the prevention and delay of CKD. As rice is the leading staple food in many countries, low-protein rice should be considered for dietary therapy. Brown rice, in particular, contains many functional factors for health, so that low-protein brown rice could provide additional benefits for CKD patients.

**Methods:** Four steps are necessary to produce low-protein brown rice of high quality: selection of rice cultivar, surface treatment of brown rice with a penetrating enzyme solution, protein extraction, and hygienic packaging. We developed a new low-protein brown rice (LPBR) product using protease treatment and *Lactobacillus plantarum* fermentation.

**Results:** LPBR maintained the high energy content of rice. While it provided the lowest protein content possible (0.2 g/100 g of boiled rice), almost no potassium (0.5 mg/100 g boiled rice), and less than 1/5 of usual phosphate contents (17 mg/100 g boiled rice). In addition, toxic metals such as antimony and cadmium were not present. Like common brown rice brands, the new product had high dietary fibers (1.0 g/100 g boiled rice), γ-oryzanol (6.3 mg/100g boiled rice), and antioxidant activity (300 ORAC Unit).

**Conclusion:** A low-protein diet has been known to be effective for the prevention of CKD. LPBR could provide enough energy with low protein, low potassium, low phosphate. Furthermore, the remaining dietary fibers, γ-oryzanol, and antioxidant activity could have sound effects to stabilize intestinal microbiota, short-chain fatty acids, and innate immunity.

**Keywords:** Low-protein Brown Rice; *Lactobacillus;* Enzyme Solution; Chronic Kidney Disease; Dietary Therapy; Himeiku-83

## Abbreviation

LPBR: Low Protein Brown Rice.

## Introduction

The global burden of chronic kidney disease (CKD) is an essential public-health matter because of its progression to end-stage and the resulting extra healthcare costs [1-5]. The prevalence of CKD is estimated to be 8–16% worldwide, and it is linked to other major lifestyle-related diseases, such as diabetes mellitus, cardiovascular diseases, and hypertension [6-10]. CKD is defined as a reduced glomerular filtration rate (GFR), an increased urinary albumin excretion, or both [1,2]. Recently, diabetes mellitus has become the most common cause of CKD [6]. Diabetic kidney disease (DKD) occurs in 20% to 40% of patients with diabetes mellitus. It is the leading cause of CKD and ESRD in the United States [9] and Japan.

The economic costs of CKD are expected to reach enormous amounts, and strategies to delay the onset or progression are urgently required. Recently the occurrence of CKD has been increasing among the elderly. The cumulative risk of hemodialysis until age 80 years is 1/50 in males and 1/100 in females [10]. Therefore, it is imperative to delay the conditions that make hemodialysis necessary to decrease medical costs and improve patients' quality of life. Dietary control is essential. The recommended dietary daily intake of proteins is 0.8 g/kg body weight, representing an average of 60 g/day for men and 50 g/day for women [11].

A Cochrane review has concluded that we would set the optimal protein intake for CKD patients at 0.4-0.6 g/kg body weight [12]. According to the Japanese Guidelines of the Kidney Association for CKD, it is 0.6-0.8 g/kg body weight, and the recommended protein intake during hemodialysis is 1.2–1.5 g/kg body weight [13]. The amount of protein intake to suppress CKD progression is still in debate. We propose that 0.5 g/kg body weight is adequate, in line with Cochrane's recommendation. Unique tailor-made design is necessary for advanced CKD patients.

As rice is the staple food in Japan, low-protein white rice with less than 1/2 protein content has been recommended for some patients [14]. However, the taste and texture of the initial preparations were not satisfactory. Brown rice contains six times more dietary fibers in weight than polished rice and more minerals and vitamins. However, the protein content was still high (6-8%) and was covered by a strong wax layer. In addition, brown rice is usually contaminated with aerobic spores and other germs inside the grain epidermis. These represent technical obstacles to the preparation of low-protein brown rice (LPBR) [15]. We have established that the selection of four steps was essential: rice cultivars, proper grain surface treatment, selection of enzyme solution to extrude rice proteins, and packing method. We challenged to produce an ideal LPBR, characterized by low protein, low potassium, low phosphate, remaining dietary fiber, γ-oryzanol and anti-oxidant activity, by the consortium on the platform of the Ministry of Agriculture, Forestry and Fisheries.

## Materials and Methods

### Rice

Rice proteins are constituted by easily digestible glutelin and globulin and indigestible prolamin [16,17]. So far, low-gluterin rice, such as the Shunyo and LGC-jun varieties developed in Japan, have a taste that disturb their use in an ordinary diet. A newly established species of paddy rice, "Himeiku-83" (Ehime Research Institute of Agriculture, Forestry and Fisheries), was selected for this project. This new variety was a hybrid between "Chugoku-188" as mother and "Himeiku-71" as the father. Chugoku-188 had both the Lgc1 gene, which decreased glutelin, and the glb1 gene, which deprived globulin (Figure 1). As compared with Shunyo and LGC-jun, Himeiku-83 had a better taste and good grain qualities. The taste evaluation score by the Japan Grain Inspection Association was high. The taste of Himeiku-83 was as delicious as Koshihikari. The yield of Himeiku-83 was higher than that of Hinohikari. The protein contents of brown rice are 6.3% - 7.0%. The variety registration was applied on November 9, 2020 [18].

**Figure 1:** SDS-PAGE pattern of proteins in brown rice. Himeiku-83 shows less amount of easy digestible glutelins and globulin.

### Pretreatment and protein extraction

The purpose of the polishing process was to damage the wax layer of the surface of brown rice [19]. It is intended to perform less than 1% refinement at the Mitsuhashi Co. Ltd. to make fine scratches on the surface. The surface of brown rice showed fine scratches, and the rice seemed to be glassy white.

### Protein extraction

**Figure 2:** Serial steps to produce a low-protein brown rice.

Extraction of protein included two-step of fermentation. The sterilized rice was immersed in a solution containing the proteolytic enzyme "Protease M (Amano) G" and Lactobacillus plantarum as primary fermentation agent under anaerobic conditions.

For processing into packed rice, trays containing one serving (99 g) of fermented brown rice were steamed for 10 minutes in a steamer, added 58 ml of hot water (70ºC), and steamed again. After cooking, a seal was put in the hot state.

As it is known that heat-stable bacteria (such as *Bacillus sp.*) are present near the aleurone layer of brown rice [20], the epidermis (pericarp and seed coat) was sterilized and washed for damaged brown rice. In the sterilization and cleaning process, polished brown rice was placed in 2% aqueous citric acid solution, heated to 70ºC, and stirred to remove impurities, germs, and stains adhering to the surface. Quality control includes three cycles of detection of contamination with heat-resistant bacteria in the processed LPBR packs. The final products showed the absence of living bacilli, coliforms sp., *Bacillus cereus* sp., heat-resistant aerobic, and anaerobic bacilli [21].

Extraction of protein included two-step of fermentation (Figure 2). After sterilizing and cleaning the surface by citric acid solution, the sterilized rice was immersed in a solution containing the proteolytic enzyme "Protease M (Amano) G" and *Lactobacillus plantarum* as primary fermentation agent under anaerobic conditions [15]. The rice was fermented for three days at 40ºC. After draining, a secondary anaerobic fermentation was performed by the same *Lactobacillus*-containing solution for 1.5 days at 40ºC. Then it was washed with water to remove surplus, and after washing with cold water again, we drained it again.

After the fermentation process, a removal cleaning process was performed, involving draining the secondary fermentation. After that, wash with running water for 2 hours, and fermentation products such as organic acids and proteins generated during fermentation were washed away with decomposing substances and residual lactic acid bacteria. After the removal and washing treatment, the protein content was measured by the Dumas method.

### Packing

For processing into packed rice, trays containing one serving (99 g) of fermented brown rice were steamed for 10 minutes in a steamer, added 58 ml of hot water (70ºC), and steamed again. After cooking, a seal was put in the hot state. Each package was sterilized by immersion in a constant temperature water tank at 85ºC for 25 minutes and then cool. We kept the pH of the final product of the packed rice at 4.25 by spraying 0. 1% gluconic acid [21].

### Measurement

The boiled and immediately frozen rice was sent to the Nippon Food Analysis Center, Tsukuba, Japan, for nutrients measurement [22], such as energy, major nutrients, vitamins, minerals, and γ-oryzanol. These were measured according to the Ministry of Agriculture, Forestries and Fisheries guidelines.

SUNTEC Research Laboratories measured the following; vitamin B1, minerals (calcium, phosphate, iron, sodium, potassium, magnesium, zinc, copper, selenium, manganate), and 28 amino acids. Antimony and cadmium were assessed to confirm safety [23]. These were single measurement from a single rice pack. Cooked brown rice and LPBR were compared and residual percent after protein extraction was calculated.

Antioxidant activity was measured by a modified method of Oxygen Radical Absorbance Capacity (ORAC) by the AOU Research

Association. ORAC measurement used 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as a standard antioxidant capacity sample and was expressed as Trolox equivalent [24]. Water-soluble and lipid-soluble AOU (ORAC) were separately measured [25-27].

## Results

### Major nutrients and antioxidant activity in LPBR

Macronutrients and other ingredients are shown in table 1. Among the several different ways Biotech2020 showed the best results (data not shown). A slight reduction in energy source was caused by immersion of cooking water, which made soft palatability like polished white rice. The remaining protein was 15% (0.2 g/100 g boiled rice). Ninety nine percent potassium and 85% phosphate were removed. Dietary fibers and γ-oryzanol remained two-third of brown rice (1.0 g and 6.3 mg /100 g boiled rice, respectively). Water-soluble fibers seemed to be more easily resolved than insoluble dietary fibers during the processing. As for other micronutrients, the reduction of minerals was characteristic in LPBR. In addition to the potassium and phosphate change, magnesium and manganese remained about only 5%. Certain amount of zinc and calcium remained a little more. Toxic As and Cd were not present.

| Item | Unit | BR | LPBR | % remained | Item | Unit | BR | LPBR | % Remained |
|---|---|---|---|---|---|---|---|---|---|
| Energy | kcal/100g | 244 | 156 | 63.9 | Vitamin B1 | mg/100g | 0.12 | <0.01 | nd |
| Water | g/100g | 40.7 | 62.2 | 152.8 | Arg | mg/100g | 240 | nd | 0 |
| Carbohydrate | g/100g | 57.1 | 36.3 | 63.6 | Lys | mg/100g | 110 | nd | 0 |
| Sugar | g/100g | 55.6 | 35.3 | 63.5 | His | mg/100g | 77 | nd | 0 |
| Protein | g/100g | 1.3 | 0.2 | 15.4 | Phe | mg/100g | 140 | nd | 0 |
| Lipid | g/100g | 1.9 | 1.3 | 68.4 | Tyr | mg/100g | 120 | 1 | 0.9 |
| Ash | g/100g | 0.1 | 0.1 | 100.0 | Leu | mg/100g | 220 | nd | 0 |
| Potassium | mg/100g | 85.3 | 0.5 | 0.6 | iLeu | mg/100g | 110 | nd | 0 |
| Phosphate | mg/100g | 115 | 17 | 14.8 | Met | mg/100g | 66 | nd | 0 |
| Dietary fiber | g/100g | 1.5 | 1.0 | 66.7 | Val | mg/100g | 160 | nd | 0 |
| γ-oryzanol | mg/100g | 10.4 | 6.3 | 60.6 | Ala | mg/100g | 160 | nd | 0 |
| NaCleq | g/100g | 0.0041 | 0.003 | 73.2 | Gly | mg/100g | 140 | nd | 0 |
| Na | mg/100g | 2.5 | 1.8 | 72.0 | Pro | mg/100g | 130 | nd | 0 |
| K | mg/100g | 85.3 | 0.5 | 0.6 | Glu | mg/100g | 470 | nd | 0 |
| Ca | mg/100g | 6 | 6 | 100.0 | Ser | mg/100g | 140 | nd | 0 |
| Mg | mg/100g | 47.5 | 2.2 | 4.6 | Thr | mg/100g | 100 | nd | 0 |
| P | mg/100g | 115 | 17 | 14.8 | Asp | mg/100g | 260 | nd | 0 |
| Fe | mg/100g | 0.4 | <0.1 | nd | Try | mg/100g | 42 | 2 | 4.8 |
| Zn | mg/100g | 0.76 | 0.12 | 15.8 | Cys | mg/100g | 58 | nd | 0 |
| Cu | mg/100g | 0.1 | <0.1 | nd | Antioxidant | | | | |
| Mn | mg/100g | 0.83 | 0.05 | 6.0 | ORAC total | umole TE/g | 350 | 300 | 85.7 |
| $As_2O_3$ | <0.1ug/g | 0 | 0 | 0 | ORAC hydrophilic | umole TE/g | 150 | 100 | 66.7 |
| Cd | <0.05 ug/g | 0 | 0 | 0 | ORAC lipophilic | umole TE/g | 200 | 200 | 100 |

**Table 1:** Major and minor nutrients of Low-Protein Brown Rice.

BR; Brown rice before protein extraction. Biotech2020 used enzyme extraction and Lactobacillus fermentation. LPBR of Biotech shows the lowest protein contents. Remaining potassium is only 0.6%, and that of phosphate is 14.8%. While about two third of dietary fiber and γ-oryzanol are present. Antioxidant activity remains 85%.

Degree of remaining minerals is various. Ca, Na, Zn, then Mn and Mg remain, but Fe and Cu are lost. Almost all amino acids are lost, except Try and Tyr.

Vitamins were also solved Vitamin B1 remained only as trace amounts. Only a small amount of tryptophan and tyrosine were detected among free amino acids.

Antioxidant activity of brown rice and LPBR remained 3-4 units in the boiled rice, mostly water-soluble AOU-L. AOU-P remained in the current LPBR. Polished rice did not show antioxidant activity at all [28].

Benefits of low protein rice and benefits of brown rice are conceptually shown in figure 3. In addition to the remaining carbohydrate (energy source), low protein, low potassium, low phosphate character of low protein rice, presence of dietary fiber, γ-oryzanol and antioxidant activity was the characteristic biomarkers of brown rice.

**Figure 3:** Comparison of nutrients in brown rice, LPBR and polished white rice.

Benefits of low protein rice are high energy content, low protein, low potassium and low phosphate. In addition, benefits of brown rice character are dietary fiber, γ-oryzanol and antioxidant activity.

## Discussion

### Effect of *Lactobacillus* for LPBR production

The combination of four steps was the optimal process to produce LPBR. In *Lactobacillus* fermentation, the ability to produce lactic acid was high, and the pH was rapidly reduced in the early stage of fermentation, resulting in an acidic environment. In the optimum pH range, the activity of protease increased, proteolysis was promoted, and a sharply decreased pH suppressed the growth of other bacteria.

The fermentation tank became anaerobic at an early stage when lactic acid bacteria enter the bran part. Because of carbon dioxide production, a gap appears between the bran and the endosperm, which was easily filled by enzymes and lactic acid bacteria. Lactic acid fermentation and proteolysis treatment at the site were efficient. Further ethanol production suppressed the growth of residual aerobic bacteria that were relatively resistant to lactic acid [22,29].

### Nutrients evaluation of LPBR

Early intervention through a low-protein diet is adequate to decrease proteinuria in CKD [29-33].

LPBR could make it easier to keep a regimen of 30 g protein/day with enough energy source intake [34]. In addition to decreased protein intake, CKD patients need to reduce their phosphorus and potassium intake [35]. Energy source intake was important to keep the efficiency of protein. We employed a simple estimate of necessary energy by 0.4 x (kg body weight) unit, where one unit was 80 kcal [34].

LPBR meets all of these requirements as a staple food for CKD patients. Additional benefits should be obtained for the disease by dietary fibers, γ−oryzanol, ferulic acid, and antioxidant activity. Almost no potassium and low phosphate in LPBR were beneficial for the patients to prevent hyperkaliemia and hyperphosphatemia [35,36].

A pack of LPBR contains 150 g LPBR (234 kcal), while the protein content is only 0.3 g. When a patient eats three packs a day, he or she could intake 702 kcal and only 0.9 g protein in total. Potassium was 2.25 mg, phosphate was 76.5 mg, and almost no NaCl. In addition, the dietary fiber contents were 4.5 g, γ−oryzanol 28.4 mg, which could stabilize the intestinal environment and intestinal bacteria [37,38]. The antioxidant ability was 1350 µmole TEQ which activity was one-fourth of daily intake. Many phytochemicals contain antioxidants, which protect from damage caused by free radicals. The U.S. Department of Agriculture measured the antioxidant capacities of 326 food items. In fruits and vegetables, the ORAC values for the hydrophilic fractions (H-ORAC) were typically much higher than those of the lipophilic fractions (LORAC) [24]. LORAC remained in the current LPBR because the wax layer was protected. Consumption of rice is about 500 g a day as a staple food in daily meals should prevent carcinogenesis and diseases caused by free radicals [27].

These characteristics make LPBR the ideal staple food for CKD patients. In combination with soy protein for side dishes, it could help to achieve a tasty low protein diet [39].

## Conclusion

A new low protein brown rice (LPBR) had both benefits of low-protein rice and brown rice itself, namely: dietary fibers, γ–oryzanol, and antioxidant activity. The palatability is comparable to polished white rice. CKD is increasing worldwide, and recently it happened in an aging society and the diabetic complication and end-stage glomerulonephritis. A low-protein diet is the most effective intervention for the prevention of CKD. LPBR could provide enough energy with low protein, low potassium, low phosphate, and brown rice's health benefits by dietary fiber, γ–oryzanol, and antioxidant activity for CKD patients. LPBR could be available for patients with renal insufficiency at any stage.

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## Author Contribution

All authors proceeded the work in consortium.

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## Conflict of Interest

The authors had no conflict of interest to declare.

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**Volume 5 Issue 8 August 2021**