Effects of Germination on Nutrient Composition of Long Grain Rice and its Protein Physico-Chemical and Functional Properties

Dinara D. Tortayeva¹, Navam Hettiarachchy¹, Ronny Horax¹, Satchi Eswaranandam¹, Alok Jha²

¹Department of Food Science, University of Arkansas, 2650 N. Young Ave, Fayetteville, AR 72704, USA
²Center for Food Science and Technology, Banaras Hindu University, Varanasi, India

Corresponding author: Navam S. Hettiarachchy, 2650 N. Young Ave., Fayetteville, AR 72704, Phone: 479-575-4779, Fax: 479-575-6936, E-mail: nhettiar@uark.edu

Citation: Navam S. Hettiarachchy, et al. (2014) Effects of Germination on Nutrient Composition of Long Grain Rice and its Protein Physico-Chemical and Functional Properties. J Food Nutr 1: 1-9

Abstract

Germination can enhance the nutritional and nutraceutical value of whole grain rice. Rough long grain rice was germinated for 7 days. Samples were collected from day 1 to 7 and dried to 12% moisture content. Sample collected at 0 day was included as control (non-germinated rice). The germination process caused a decrease in lipid content from 4.4% to 3.7%. The proteins isolated from non-germinated and germinated rice samples for 1, 3, and 5 days separated into 7 bands that ranged in molecular size from 115 kDa to 21 kDa. High-density bands were observed at 26 kDa and at 33.5 kDa and some at 56 kDa (control and 1st day of germination). However, the disappearance of high molecular size bands (around 56 and 26 kDa) observed at 6 and 7 days and appearance of low dense molecular size bands with the progression of germination may be explained by proteases activity during germination. This property can be used to prepare digestible proteins in infant formulations and food products for the elderly. The surface hydrophobicity of protein isolate from control/non-germinated rice was 74,672, and germinated at day 1 to 7 days were 22,793; 23,966; 20,903; 21,411; 23,895; 27,614; and 20,110, respectively. Emulsifying and foaming activities of the proteins derived from germination were significantly lower than that of the control. This could be due to the size and the orientation of amino acids in the proteins/peptides that could not confer these properties. However, these small size proteins/peptides derived from germination could provide comfortable proteins to infants, children and elderly.

Keywords: Long grain rice; Germination; Nutrient; Physico-chemical property; Functionality; Protein

Introduction

Efforts are being made by the food industry to enhance the use of whole grain rice and to create new products. The nutritional benefits of germinated brown rice (GBR) have attracted the attention of consumers [1]. Rice production worldwide has increased from about 200 million tons of rough rice in 1960 to more than 690 million tons in 2012, and milled rice is about 68% of rough rice by weight [2]. The top three rice producers are China (29.4% of world production), India (21.8%), and Indonesia (8.5%) as of the year of 2012; the United States accounts for 1.3% of global rice [2].

The U.S. Food and Drug Administration (FDA) allowed 100% whole grain rice, to use the official claim and carry the statement that “Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat and cholesterol may reduce the risk of heart disease and some types of cancers” [3].

Germinated brown rice (GBR), produced by soaking rough rice grain followed with steaming can be easily cooked after de-hulling and provides a softer texture [4]. The soft texture is due to the physiological activity of rice itself and activities of various enzymes which make it easy to cook [5]. During germination, dormant enzymes activate and changes occur from the process of converting the stored carbohydrates, lipids, and proteins into the energy required for the germination process. The most notable result of these changes is the production of new generated nutritionally valuable compounds such as free amino acids and peptides [6].

Germinated rice is effectively digested and assimilated in the body and considered to be a functional food with a high content of nutrients that are beneficial to the human body. It contains γ-aminobutyric acid (GABA), ferulic acid, dietary fiber and various other nutrients [7]. Okada et al. reported that intake of GABA for 8 weeks
suppressed blood pressure and improved sleeplessness, and an autonomic disorder observed during the menopausal or presenile period [8]. Hagiwara et al. found that pre-germinated brown rice (GBR) may be effective in preventing of diabetic vascular complications such as ischecmic heart disease, and the increase in blood glucose concentration [9]. It also reduces the risk of developing some cancers, helps to manage weight, and has antiallergenic properties [10 - 12]. In addition, functional properties of the protein derived from germinated brown rice needs to be investigated for food application.

The objectives of this research were to germinate rough long grain rice, and to investigate the changes that take place in the nutrients during germination, and to study the physical and functional properties of rice protein during rice germination.

Materials and Methods

Materials

Commercial rough rice (long grain variety, crop 2005) was provided by Riceland Foods Inc. (Stuttgart, AR, USA). All chemicals for protein, lipid and mineral content determinations, protein extraction, surface hydrophobicity, emulsifying and foaming properties were purchased from VWR International, Inc. (Suwanee, GA, USA) and Sigma Chemical Co. (St. Louis, MO, USA). Standards, sodium eluent (pH 3.15, pH 7.40), sodium sample diluent (pH 2.20), and TRIONE ninhydrin reagent for amino acid analysis and sodium ion exchange to separate amino acids were purchased from Pickering Laboratories, Inc. (Mountain View, CA, USA). Electrophoresis reagents, SDS-PAGE standard composed of trypsin inhibitor (21.5 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), serum albumin (66.2 kDa), phosphorylase B (97.4 kDa), β-galactosidase (116.3 kDa), and myosin (200 kDa), and Coomassie Brilliant Blue R-250 dye were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA).

Methods

Long grain rice germination

Nine kilograms of rough long grain rice was cleaned, washed thoroughly in water and soaked in water 1:2 w/v for 24 hours at ambient temperature. The drained rice was placed in the cotton bags (48 cm x 56 cm) and incubated at ambient temperature (30 °C) for 36 hours for the germ to develop. After the incubation period, rice was spread on two-layer trays (65 cm x 45 cm x 2.5 cm). Trays were placed one above another. The upper tray had holes in it to give the germs grow. The lower tray contained the water. Approximately 600 grams of germinated rice was collected every day starting from the first day after the incubation period up to 7 days. On the 7th day, the shoots and roots of rice were fully developed. The samples were dried to 12% moisture content (MC) for further analysis. The germination procedure was repeated three times in a completely randomized design. The non-germinated rough rice sample (0 day) was used as a control. Germinated rice was dried to reach a MC of 12% (is ideal for avoiding microbial growth) in a walk-in drying chamber (Parameter and Generation Control, Black Mountain, NC, USA); this MC was determined by measuring 50 kernels of the rice sample using an individual kernel moisture tester (CTR-800E, Shizuoka Seiki Co. Ltd., Shizuoka-ken, Japan).

Preparation of flour from germinated rice

Dried non-germinated and germinated rice samples were processed (including milling fractions of rice: hull, bran, endosperm, germ) into flour using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO, USA) and passed through an 80-mesh sieve to obtain a uniform particle size.

Chemical composition of rice before and after germination

Chemical composition of NGRF (non-germinated rice flour), and GRF (germinated rice flour) were determined using the Official Methods of the American Association of Cereal Chemists for moisture, lipid, ash, and protein. The MC were determined using an air dry oven at temperature 130 °C for 2 hours; lipid contents were determined by a soxhlet method using petroleum ether as the extraction solvent; ash contents were determined as the residue remaining after incineration using an electric muffle furnace at 600 °C for 4 hours [13]. The protein contents were determined by method 46-08 using an Automatic Kjeltcemc 2300 Analyzer Unit (Kjel-Foss Tecator, Hoganas, Sweden) after digestion for 1 hour at 420 °C using a Kjeldahl 2006 Digestor with Kjeldahl® tablet as the catalyst [14]. Nitrogen conversion factor of 6.25 was used to calculate the protein content. Carbohydrate contents were determined by subtracting the sum percentage of moisture, protein, lipid and ash contents.

Preparation of rice protein isolate

One hundred grams of NGRF and GRF were defatted with 400 mL hexane. The defatted NGRF and GRF were dried under a hood at ambient temperature for 24 hours to evaporate residual hexane from the flour. Rice protein from defatted NGRF and GRF were extracted by stirring 100 grams of the defatted flour with 400 mL of distilled water (pH of the slurry was adjusted to 10.0 using 3M NaOH) at 20 °C for 3 hours. The supernatant was collected by centrifugation at 15,300 x g for 10 min. Collected residue was re-extracted (second extraction) in 150 ml distilled water (pH 10.0) for 1 hour with stirring. Combination of the supernatants from first and second extractions were isoelectrically precipitated at pH 4.5 and kept at 4 °C overnight. The precipitate was recovered by centrifugation at 15,300 x g for 5 minutes to obtain rice protein isolate (RPI). The RPI was refined by washing with deionized water (pH 4.5) and centrifugation at 15,300 x g for 15 min. The refined RPI in small amount of water was adjusted to pH 7.0, and freeze dried (Genesis SQ Freeze Dryer VirTis, SP Industries, Inc., Gardiner, NY, USA).
Amino acid analysis

Amino acid analysis of the RPIs was performed according to an official standard procedure (method 994.12, 17) [15]. RPI was treated with performic acid for 16 h prior to hydrolysis for oxidation of cystine and methionine to cysteic acid and methionine sulfone, respectively. After oxidation, sodium metabisulfite was added to decompose performic acid. The amino acids of the oxidized RPIs were liberated by hydrolysis with 6M HCl-phenol solution for 24 hours at 110-120 °C. The obtained hydrolysates were evaporated at 60 °C to dryness using a rotary evaporator. Aliquots were made by solubilizing the evaporated hydrolysates with sodium citrate buffer (pH 2.2). Norleucine standard solution was added into the hydrolyzed samples as an internal standard and mixed. The solutions were made up to 50.0 mL with the same buffer and passed through a 0.2-µL membrane filter into autosampler tubes. The solutions (20.0 mL) were injected and analyzed with a 126AA Solvent Module connected to a Spherogel™ IEX High Performance Sodium column (Beckman Instruments, Inc., Palo Alto, CA, USA). Flow rate of the detector was set up to 0.67 mL/min (0.44 mL/min for buffer solutions and 0.23 mL/min for ninhydrin solutions) using sodium citrate buffer (pH 3.3, 4.3, and 6.3) as eluents. The separated individual amino acid components were detected at 570 nm absorbance. Standard amino acid profiles were used to identify and quantify the amino acids by comparing the peak profiles. The amino acid content was expressed as mg/g rice.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method developed by Laemmli was used to identify the approximate molecular weights of the protein fractions from RPIs [16]. The method was applied using gel made from 4% stacking gel and 12% separating gel in an SDS-Tris-Glycine discontinuous buffer system. Protein solutions (5 μg protein/μL) were prepared in reducing sample buffer (62.5mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.05% bromphenol blue) for loading onto the gel. Electrophoresis was run at a constant current (45 mA) for approximately 40 min in an electrode buffer solution (pH 8.3) by a power supply model 3000 XΙ (Bio-Red Laboratory, Richmond, Ca., USA). The gel was stained with 0.1% Coomassie Brilliant Blue R-250 in a solution containing 10% acetic acid, 40% ethanol and 50% water and de-stained in the same solvent excluding Coomassie Brilliant Blue R-250. The molecular mass standard (composed of trypsin inhibitor, 21.5 kDa; carbonic anhydrase, 31 kDa; ovalbumin, 45 kDa, serum albumin, 66.2 kDa; phosphorylase b, 97.4 kDa; β-galactosidase, 116.5 kDa; and myosin, 200 kDa) from Bio-Rad used was also run along with protein samples.

Surface hydrophobicity of proteins determination

Surface hydrophobicity (SH) was determined based on the method developed by Hayakawa and Nakai called hydrophobic fluorescence probes using 1-anilino-8-naphthalene sulfonate (ANS) reagent [17]. Four milliliters of a series of rice protein solutions (prepared in 0.01 M phosphate buffer, pH 7.0; protein concentrations for the control ranged from 0.0005 to 0.01% and for the germinated rice protein isolates were from 0.0015 to 0.02%) were added with 10 µL of 8mM ANS in 0.01M phosphate buffer (pH 7.0) and vortexed. Fluorescence intensities of the protein solutions were read using a spectrophotometer (Model RF-1501, Shimadzu, Kyoto, Japan) at 390 nm of excitation and 470 nm of emission. SHs of the rice proteins were expressed as indexes of the coefficient values of the regression equation obtained from the linear regression of fluorescence intensity vs. protein concentration.

Emulsifying Properties

Emulsifying activity (EA) and emulsion stability (ES) of rice proteins before and after germination were determined by the turbidimetric methods developed by Pearce and Kinsella [18]. A suspension of 6 mL 0.1% protein solution (w/v, pH 7.0) and 2.0 mL of corn oil were made by homogenization for 1 min using a sonicator at setting 6 (Virtishear Tempest, The Virtis Co., Gardner, NY, USA). Fifty microliters of this emulsion were directly pipetted into 5 mL of 0.1% SDS (w/v) at 0 and 10 min after homogenization. The solution was thoroughly mixed and absorbance of this solution was read at 500 nm using a spectrophotometer (Model UV LVE-1601, Shimadzu, Kyoto, Japan). Absorbance read at 0 min (T₀) was expressed as EA of protein, while ES was calculated as follows: ES = T₀ x ΔT/ΔT, where ΔT is the decrease in turbidity (absorbance) of T₀ in the time interval Δt (10 min), and T₀ is the absorbance of the emulsion right after homogenization.

Foaming Properties

Foaming capacity (FC) and foaming stability (FS) of the protein isolates were measured by a method established by Kato et al. [19]. Air (200 cm³/min) was introduced into 5 mL of protein solution (0.2% w/v in 0.05 M phosphate buffer, pH 7.0) in a graduating cylinder for 15 s. The volume (in mL) of foams formed was determined immediately after air introduction by directly reading from the cylinder and this volume was considered as the volume of foam at 0 time (soon after introduction stopped). The nutrient composition of non-germinated and germinated rice and physico-chemical, functional properties of the rice protein isolates were analyzed by one-way ANOVA using JMP (JMP 5 Starter Statistics 2002). All values were reported as means of three determinations; the data were presented as mean ± standard error (SE). The protected least significant difference (LSD) procedure was used to compare the means (when the
Results and Discussion

Proximate composition of non-germinated and germinated long grain rice

The chemical composition of rice before and after germination is shown in Table 1. All results were compared to the control/non-germinated rice. The MC (P < 0.0001) and ash (P = 0.0229) significantly decreased over time while the percentages of protein (P = 3910), lipid (P = 0.1318) and carbohydrate (P = 0.6246) statistically showed no significant variance throughout the germination period. The protein after 1 day of germination tended to decrease indicating nitrogen breakdown and translocation to the embryonic tissues. Protein content at the seventh day of germination showed slight increase (although not significant) in protein content from 7.3% to 7.5%. An increase in protein content may be attributed to a passive variation due to decrease in the carbohydrate compound used for respiration. Hamad and Fields reported an increase in crude protein in germinated rice [20]. Most of the stored protein of cereals is located in the aleurone grains (bodies) of the aleurone layer, and in the protein bodies of the endosperm. Aleurone layer releases several different proteinases which mobilize the reserve proteins of the starchy endosperm [21].

The lipid decreased from day 3 (4.2 g/100 g) to 7 (3.7 g/100 g) day of germination; however the decrease was not significantly different (P = 0.1318). Similar trend was observed by Bau et al. on soya bean seeds [22], Fernandez et al. on maize [23], and Gamel et al. on amaranth seeds [24]. Rice lipids mainly consist of triacylglycerol and occur in the spherosome located in the aleurone cells and embryo which are responsible for deterioration of rice flavor. Triacylglycerol stored in the lipid body of the rice grain could have been mobilized during germination to provide energy for embryo growth and provide energy required for protein synthesis [25].

Juliano [26], Eggum et al. [27], and Pedersen and Eggum [28] reported the ash content of rough rice in the range of 2.9-5.2%. Rice germinated for 5 days showed the highest percentage of the ash content (P = 0.0229) of 7.2% in comparison with the control 5.4% (P < 0.05). The increase in ash content is attributed to an increase in thickness of the hull.

Amino acid composition

Amino acid composition of non-germinated and germinated long grain rice are given in Table 2. Rice protein is considered to have fairly good balance of essential amino acids; however, the proportions are not ideally balanced. Juliano [26], Eggum et al. [27], and Pedersen and Eggum [28] reported “best estimate” of the typical amino acid composition of rough rice and its milling fractions. The control and germinated rice at 1 to 7 days had similar amino acid pattern. Cysteic acid content of germinated rice from day 1 to 7 was not significantly different (P = 0.1018). Control has the highest cysteic acid content of 1.8 mg/g rice. Cysteine is classified as a non-essential amino acid. In rare cases, cysteine may be essential for infants, the elderly, and individuals with certain metabolic disease. Cysteine is an important source of sulfide in human metabolism and can usually be synthesized by the human body under normal physiological conditions if a sufficient quantity of methionine is available [29].

Glutamine and asparagine are converted to form glutamic and aspartic acids. Asparagine content dropped at 1 day of germination (5.3 mg/g rice) and increased at 7 days of germination (6.5 mg/g rice) which was significantly different (P = 0.0030). Asparagine is required by the nervous system and it, also, plays an important role in the synthesis of ammonia. Serine is important in the catalytic function of many enzymes and the precursor to several amino acids, including glycine, cysteine, and, in bacteria, tryptophan [30]. Tryptophan is not present due to decomposition by the hydrolysis with HCl.

Threonine content at 1 day of germination (1.9 mg/g rice) showed no significant difference with the control (1.5 mg/g rice) and with the germinated rice at 7 days (P = 0.0685). Threonine, together with serine (P = 0.1696) and tyrosine, is one of three proteinogenic amino acids bearing an alcohol

---

**Table 1:** Proximate composition of long grain rice during germination (g/100 g, dry weight basis)

| Germination days | Moisture* | Protein | Lipid | Ash | Carbohydrate |
|------------------|-----------|---------|-------|-----|--------------|
| 0 day (control)**| 10.9 ± 0.1a | 7.3 ± 0.2 | 4.4 ± 0.1 | 5.4 ± 0.7bc | 83.0 ± 0.2 |
| 1                | 7.6 ± 0.2b | 6.5 ± 0.4 | 4.4 ± 0.4 | 6.1 ± 0.3abc | 83.8 ± 0.7 |
| 2                | 7.6 ± 0.2b | 6.5 ± 0.9 | 4.4 ± 0.1 | 5.0 ± 0.2c | 83.7 ± 0.9 |
| 3                | 8.2 ± 0.1b | 6.6 ± 0.3 | 4.2 ± 0.2 | 5.2 ± 0.1c | 84.0 ± 0.5 |
| 4                | 7.4 ± 0.2bc | 6.7 ± 0.1 | 4.2 ± 0.2 | 5.6 ± 0.5bc | 83.8 ± 0.2 |
| 5                | 6.4 ± 0.2cd | 6.8 ± 0.1 | 3.4 ± 0.2 | 7.2 ± 0.4a | 84.4 ± 0.3 |
| 6                | 6.5 ± 0.7cd | 7.3 ± 0.2 | 3.5 ± 0.7 | 6.4 ± 0.8ab | 83.9 ± 0.5 |
| 7                | 6.0 ± 0.4d | 7.5 ± 0.2 | 3.7 ± 0.3 | 5.9 ± 0.2bc | 83.7 ± 0.2 |
| P-value          | < 0.0001  | 0.391   | 0.1318 | 0.0229 | 0.6246       |

*Values are means ± SE of triplicate analysis; mean values followed by the same letters in the same column are not significantly different (P < 0.05).

**Control is non-germinated long grain rice.
was slight lower than that of the germinated rice at 7 days (2.2 mg/g rice) but were not significantly different in comparison to the other germinated rice samples (P = 0.9389). Along with valine, leucine and isoleucine were branched-chain amino acids. Methionine content of the control was 3.2 mg/g rice and at 7 days of germination was 3.0 mg/g rice which was not significantly different (P = 0.1122). The range of phenylalanine reported by Juliano [32] was 9.3 to 10.8 mg/g rice that made it a much higher than that obtained in the current study. The difference could be due to analytical techniques or variations in germination stage. Phenylalanine is found naturally in the breast milk of mammals. It is manufactured for their reputed analgesic and antidepressant effects. Methionine and cysteine are two sulfur-containing proteogenic amino acids. Methionine contents of the rice samples during 7 days of germination decreased in comparison to the control from 1.9 ± 0.1 to 1.4 ± 0.0 mg/g rice, which also could be due to decomposition during hydrolysis with HCl; however these were not statistically different (P = 0.3346).

There were slight differences in histidine contents between the control and 7 days of germination by 0.2 mg/g rice (P = 0.0119). Lysine content of the control and 7 days had slight differences in comparison with germinated rice at 7 days (4.2 mg/g rice). Tyrosine content (P = 0.0290) of the control (4.5 mg/g rice) was slightly higher than in germinated rice at 7 days (4.2 mg/g rice). Tyrosine is a non-essential amino acid. It is important as precursor to hormones, alkaloids, pigments, and neurotransmitters. Phenylalanine content of the control was 3.2 mg/g rice and at 7 days of germination was 3.0 mg/g rice which was not significantly different (P = 0.1122). The range of phenylalanine reported by Juliano [32] was 9.3 to 10.8 mg/g rice that made it a much higher than that obtained in the current study. The difference could be due to analytical techniques or variations in germination stage. Phenylalanine is found naturally in the breast milk of mammals. It is manufactured for their reputed analgesic and antidepressant effects. Methionine and cysteine are two sulfur-containing proteogenic amino acids. Methionine contents of the rice samples during 7 days of germination decreased in comparison to the control from 1.9 ± 0.1 to 1.4 ± 0.0 mg/g rice, which also could be due to decomposition during hydrolysis with HCl; however these were not statistically different (P = 0.3346).

There were slight differences in histidine contents between the control and 7 days of germination by 0.2 mg/g rice (P = 0.0119). Lysine content of the control and 7 days had slight differences in comparison with germinated rice at 7 days (4.2 mg/g rice). Tyrosine content (P = 0.0290) of the control (4.5 mg/g rice) was slightly higher than in germinated rice at 7 days (4.2 mg/g rice). Tyrosine is a non-essential amino acid. It is important as precursor to hormones, alkaloids, pigments, and neurotransmitters. Phenylalanine content of the control was 3.2 mg/g rice and at 7 days of germination was 3.0 mg/g rice which was not significantly different (P = 0.1122). The range of phenylalanine reported by Juliano [32] was 9.3 to 10.8 mg/g rice that made it a much higher than that obtained in the current study. The difference could be due to analytical techniques or variations in germination stage. Phenylalanine is found naturally in the breast milk of mammals. It is manufactured for their reputed analgesic and antidepressant effects. Methionine and cysteine are two sulfur-containing proteogenic amino acids. Methionine contents of the rice samples during 7 days of germination decreased in comparison to the control from 1.9 ± 0.1 to 1.4 ± 0.0 mg/g rice, which also could be due to decomposition during hydrolysis with HCl; however these were not statistically different (P = 0.3346).

There were slight differences in histidine contents between the control and 7 days of germination by 0.2 mg/g rice (P = 0.0119). Lysine content of the control and 7 days had slight
difference statistically (P < 0.0023). Histidine is considered an essential amino acid, but mostly only for children. Arginine content of the control (5.5 mg/g rice) was significantly higher than at 7 days (4.4 mg/g rice) by 1.1 mg/g rice (P < 0.0001). Arginine is a conditionally essential amino acid. It can be synthesized by the human body, and does not need to be obtained directly through the diet.

**Electrophoresis**

Electrophoretogram of rice protein isolates from non-germinated and germinated rice is presented in Figure 1. Appropriate molecular weight standards ranging from 21.5 kDa to 200 kDa were used to compare and determine the molecular weight of rice proteins from non-germinated and germinated rice. The electrophoretic profile of the control and rice protein obtained during germination for 1, 3, and 5 days showed 7 protein bands that ranged in size from 115.25 to 21.5 kDa. The high-density bands were observed at 26 kDa and at 33.5 kDa (control and 1st day of germination). However, the electrophoretic profile of rice proteins from germination at 5 to 7 days did not show any band at 26 kDa. The high-density band was observed at 23 kDa (7 days of germination). As germination progressed, the high molecular weight proteins were hydrolyzed into low molecular weight proteins. The disappearance of high molecular size bands (around 56 and 26 kDa) at 6 and 7 days and appearance of low dense molecular size bands with the progression of germination may be explained by proteases activity activated during germination.

**Surface hydrophobicity**

The surface hydrophobicity of the rice protein isolates from rice germinated for 1, 2, 3, 4, 5, 6, and 7 days compared to the control (non-germinated rice) are presented in Table 3. Linear regression of fluorescence intensity vs. protein concentration was calculated and the coefficient value of the regression equation was used as an index of protein hydrophobicity. A linear relationship between fluorescence intensity and protein concentrations was observed (r = 0.99). At 0.01% protein concentration the surface hydrophobicity values for these samples were very low. The surface hydrophobicity of the protein isolate of the control and germinated rice for 1, 2, 3, 4, 5, 6, and 7 days were 74,672; 22,793; 23,966; 20,903; 21,411; 23,895; 27,614 and 20,110 respectively. There was significant difference in surface hydrophobicity between the control and germinated rice from 1 to 7 days (P < 0.0001). However, the surface hydrophobicity values for protein from germinated rice from 1 to 7 days were not significantly different (P > 0.05).

Both polar and nonpolar amino acids are present in proteins. Folding into structures of low free energy minimizes the energy of proteins. Polar groups of proteins are maximized and nonpolar groups are minimized in the interactions with water. Therefore, native proteins tend to bury their hydro-
phobic side chain amino acids in the polypeptide chains into the core of the protein and present their hydrophilic groups at the surface. High polar interaction leads to protein-water interaction which results in increased solubility. Low surface hydrophobicity values of germinated rice from 1 to 7 days in comparison to the control were observed. Because of similar electric charges or side groups on the protein surfaces, and amino acid patterns, the surface hydrophobicity values were almost similar in the protein isolates throughout germination period.

**Emulsifying properties**

The emulsifying activity (EA) and emulsifying stability (ES) of the protein isolates from the control and of germinated rice (1 to 7 days) are shown in Table 3. Data showed that the control had relatively good emulsifying properties. Emulsifying activities among all the germinated rice throughout germination period of 7 days were significantly different (P < 0.0001). Emulsion stability of the control/non-germinated rice was 20.9 min which was not statistically significantly different from that of germinated rice from 1 to 7 days (P = 0.07). Rice germinated for 3 and 7 days had slightly lower emulsifying activity, which were 0.59 and 0.58, respectively, in comparison to others. The lower emulsifying activity may be the result of less hydrophobic residues on the surface of these proteins which disperses the droplets of oil in aqueous continuous phase of the solution.

| Germination (days) | Surface hydrophobicity* | Emulsifying activity | Emulsion stability (min) | Foaming capacity (mL) | Foaming stability (min) |
|--------------------|-------------------------|----------------------|--------------------------|-----------------------|-------------------------|
| 0 (control)        | 74,672 ± 58a            | 0.79 ± 0.06a         | 20.9 ± 0.9               | 32.1 ± 2.6c           | 17.1 ± 1.1ab            |
| 1                  | 22,793 ± 16cd           | 0.66 ± 0.02c         | 23.3 ± 3.4               | 21.6 ± 2.6d           | 17.8 ± 6.3a             |
| 2                  | 23,966 ± 16c            | 0.67 ± 0.03c         | 23.2 ± 3.2               | 31.2 ± 3.7c           | 7.3 ± 0.7de             |
| 3                  | 20,903 ± 29cd           | 0.59 ± 0.04d         | 21.8 ± 1.3               | 36.8 ± 1.8b           | 2.7 ± 0.3e              |
| 4                  | 21,411 ± 17cd           | 0.61 ± 0.04d         | 27.3 ± 7.6               | 43.9 ± 3.2a           | 3.4 ± 0.2e              |
| 5                  | 23,895 ± 21c            | 0.66 ± 0.04c         | 23.8 ± 2.8               | 18.2 ± 2.8d           | 5.8 ± 1.1e              |
| 6                  | 27,614 ± 9b             | 0.72 ± 0.01b         | 24.9 ± 1.9               | 22.6 ± 1.4d           | 11.8 ± 1.6cd            |
| 7                  | 20,110 ± 41d            | 0.58 ± 0.03d         | 20.9 ± 3.9               | 7.9 ± 0.8e            | 12.6 ± 4.5bc            |
| P-value            | < 0.0001                | < 0.0001             | 0.07                     | < 0.0001              | < 0.0001                |

*Values are means ± SE of triplicate analysis; mean values followed by the same letters in the same column are not significantly different (P < 0.05).

In a thermodynamic sense emulsions are not stable. The ability of the emulsion to slowly undergo the various processes which results in the separation of oil and water phases is considered a stable emulsion [34]. Protein from germinated rice at 4 days showed low emulsifying activity (0.61) but had the highest emulsion stability (27.3 min), and 7 days showed the lowest emulsifying activity and emulsion stability, which were 0.58 and 20.9 min, respectively. This could be due to the most exposure of hydrophobic groups of the protein. The larger the number of hydrophobic groups at the surface, the more significantly protein interacts with the oil surface.

The differences in the results obtained in this study indicated the involvement of the factors affecting emulsifying properties such as unfolding of protein, adsorption kinetics, and interfacial load, rheology of the interfacial film and decrease of interfacial tension [35]. For better understanding the emulsifying properties of proteins Kato and Nakai established a correlation between surface hydrophobicity and interfacial tension [36]. In this study, results indicated positive correlation between emulsifying properties and surface hydrophobicity. Amphiphilic proteins having high surface hydrophobicity are absorbed at the oil/water interface. Therefore, adsorbed proteins reduce the interfacial or surface tension facilitating the formation of emulsions. This correlation has been reported by Kato and Nakai, Nakai, Voutsinas et al., Hayakawa and Nakai, Aluko and Yada, Philips et al., and Petrucceli and Anon [17, 36-41].

**Foaming properties**

The foaming stability (FS) and foaming capacity (FC) of non-germinated/control and germinated rice for 7 days are given in Table 3. In foam formation proteins rapidly absorb at the interface, reduce surface tension, and form a stabilizing film around bubbles. Decrease in interfacial tension leads to increase of viscous and elastic properties of the liquid phase to form strong films. The ability to form flexible, elastic cohesive interfacial film is very important in foaming properties.

The proteins derived from germinated rice for 4 days showed the highest foaming capacity (43.89 mL) which was different in comparison with proteins from non-germinated and germinated rice for 1, 2, 3, 5, 6, and 7 days. However, rice germinated for 5 days had the lowest foaming capacity (18.2 mL) and showed significant difference with the non-germinated rice/control (P < 0.05). Volumes of the foams were measured after 3 minutes time interval. Foaming stabilities of the control and 1 day were the highest, which were 17.1 and 17.8 min, respectively. Foams obtained from 3 and 4 days were less stable than others (2.7 min and 3.4 min, respectively). The lower foam stability contributed to the lack of formation of a thick cohesive, and viscoelastic film around air. Viscoelastic film prevents the foam from collapsing. Decreasing in foaming capacity of the germinated rice protein at day 1 to 4 days may be explained by insolubility of rice proteins as germination progressed.
According to the results, proteins from non-germinated and germinated rice showed poor foaming properties. Foam formation was not significant and was unstable. After air introduction the collapse of air bubble was observed. The inability of non-germinated/control and germinated rice proteins to form stable foams is due to lack of sufficient protein-protein interaction and, therefore, a reduced cohesion \[42\]. Rice proteins were unable to form continuous intermolecular polymers enveloping the air bubbles due to its low cohesive-ness, viscosity and elasticity, and hence, poor foaming properties were observed.

Conclusion

Germination did not affect significantly on the proximate composition of rough long grain rice, except for moisture and ash contents. There were minor changes in protein, lipid and carbohydrate contents during germination but these were not statistically significant. Protein content at 7 days of germination showed slight increase in protein content which may be attributed to a passive variation due to decrease in the carbohydrate compound used for respiration. Amino acid pattern of protein from germinated rice for 7 days did not change much in comparison to the control/non-germinated rice. The limiting amino acid lysine along with glutamic had the highest contents in non-germinated rice as well as in germinated rice. The germination may reduce or possibly eliminate the allergenic proteins. The breakdown of high molecular weight proteins into low molecular weight proteins caused by germination can be beneficial in infant nutrition due to its high digestibility. High digestibility is essential to infants because of their immaturity of gastrointestinal function. Surface hydrophobicity of the control was significantly different from all germinated rice throughout germination period. Because of similar electric charges or side groups on the protein surfaces, and amino acid patterns, the low surface hydrophobicity values were almost similar in the protein isolates from germinated rice throughout germination period. The germination did not improve the emulsifying properties of rice; while, the results showed poor foaming properties of proteins for both non-germinated and germinated rice due to unstable foam formation caused by the collapse of air bubble. Further research needs to improve the food functionalities of germinated rice and that can satisfy the requirement of functional properties for food applications.

Acknowledgment

The authors would like to thank the Arkansas Rice Promotion Board for providing funds to publish the research conducted in this project.

References

1) Lahteenmaki L, Tuorila H (1998) Predicting the intention to use juice or milk in three contexts. Food Qual Preference 9: 231-236.
2) IRRI [International Rice Research Institute] (2014) World Rice Statistics Online Query Facility.
3) USA Rice Federation (2008) USA Rice Daily. May 8 2008. p1-3
4) Komatsuzaki N, Tsukahara K, Toyoshima H, Suzuki T, Shimizu N, et al. (2005) Effect of soaking and gaseous treatment on GABA content in germinated brown rice. J Food Eng 78: 556-560.
5) Kim S, Park H, Byun S (2007) Methods for preparing germinated brown rice having improved texture and cook ability without microbial contamination and germinated brown rice obtained therefrom. U. S. Patent 7217436
6) Palmiano EP, Juliano, BO (1971) Biochemical changes in the rice grain during germination. Plant Physiol 49: 751-756.
7) Zhang H, Yao H, Chen F (2006) Accumulation of α-aminobutyric acid in rice germ using protease. Biosci Biotechnol Biochem 70: 1160-1165.
8) Okada T, Sugishita T, Murakami T, Murai H, Saikusa T, et al. (2000) Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. Nippon Shokuhin Kagaku Kaishi 47: 596-603.
9) Hagiwara H, Seki T, Arita T (2004) The effect of pre-germinated brown rice intake on blood glucose and PAI – 1 levels in Streptozotocin-induced diabetic rats. Biosci Biotechnol Biochem 68: 444-447.
10) Kawabata K, Tanaka T, Murakami T, Okada T, Murai H, et al. (1999) Dietary prevention of azoxymethane-induced colon carcinogenesis with rice-germ in F344 rats. Carcinogenesis. 20: 2109-2115.
11) Lim BO, Yamada K, Cho B, Jeon T, Hwang S, et al. (2004) Comparative study on the modulation of IgE and cytokine production by Phellinus linteus grown on germinated brown rice, Phellinus linteus and germinated brown rice in murine splenocytes. Biosci Biotechnol Biochem 68: 2391-2394.
12) Lim BO, Jeon T, Hwang S, Moon JH, Park DK (2005) Phellinus linteus grown on germinated brown rice suppresses IgE production by the modulation of Th1/Th2 balance mesenteric lymph node lymphocytes. Biotechn Let- ters 27: 613-617.
13) AACc International (2000) Approved Methods of the American Association of Cereal Chemists, 10th Ed. St. Paul: The Association.
14) AACc (1990) Approved Methods of the American Association of Cereal Chemists, 8th Ed. St. Paul: The Association.
15) AOAC International (2005) Official methods of analysis of AOAC Interna- tional, 18th Ed. Gaithersburg: AOAC International.
16) Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature 227: 680-686.
17) Hayakawa S, Nakai S (1985) Relationship of hydrophobicity and net charge to the solubility of milk and soy proteins. J Food Sci 50: 486-491.
18) Pearce KN, Kinsella JE (1978) Emulsifying properties of proteins: evaluation of a turbidimetric technique. J Agric Food Chem 26: 716-723.
19) Kato A, Osaka Y, Matsudomi N, Kobayashi K (1983) Changes in emulsify- ing and foaming properties of proteins during heat denaturation. Agric Biol Chem 47: 33-37.
20) Hamad AM, Fields ML (1979) Evaluation of the protein quality and avail- able lysine of germinated and fermented cereal. J Food Sci 44: 456-459.
21) Bewley JD, Black M (1978) Development, germination, and growth. In: Physiology and biochemistry of seeds in relation to germination v. 1. Berlin, New York: Springer-Verlag, p11-28.
22) Bau M, Villaume C, Nicolas J, Mejean L (1997) Effect on germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (Glycine max) seeds. J Sci Food Agric 73:1-9.
23) Fernandez DE, Qu R, Huang AHC, Stahelin LA (1988) Immunogold localization of the L3 protein of maize lipid bodies during germination and seedling growth. Plant Physiol 86: 270-274.
24) Gamel TH, Mesallam AS, Damir, AA, Shekib LA, Linssen JP (2007) Char- acterization of amaranth seed oils. J Lipid Foods 14: 323-334.
25) Kornberg HL, Beevers H (1957) The glycoxylate cycle as a stage in the conversion of fat to carbohydrate in castor beans. Biochem Biophys Acta 26: 517-531.
26) Juliano BO (1985) The rice germination. In: Juliano BO, editor. Rice chemistry and technology, 2nd ed. St Paul: The American Association of Cereal Chemists. p774.
27) Eggum BO, Juliano BO, Maningat CC (1982) Protein and energy utiliza- tion of rice milling fractions by rats. Qualitas Plantarum-Plant Foods Human Nutr 31: 371-376.
28) Pedersen B, Eggum BO (1983) The influence of milling on the nutritive value of flour from cereal grains. 4. Rice. Plant Foods Hum Nutr 33: 267-278.
29) Hell R (1997) Molecular physiology of plant sulfur metabolism. Planta 202: 138-148.
30) Mothet J, Parent TA, Wolosker H, Brady OR, Linden JD, et al. (2000) D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U.S.A. 97: 4926-4931.

31) Lasztity R, Hidvegi M (1985) Amino Acid Composition and Biological Value of Cereal Proteins. Proceedings of the International Association for Cereal Chemistry Symposium on Amino Acid Composition and Biological Value of Cereal Proteins; Budapest, Hungary, May 31-June 1, 1983. Dordrecht / Boston / Lancaster: D. Reidel Publishing Company.

32) Juliano BO (1983) Factors affecting nutritional properties of rice protein. Trans Natl Acad Sci Technol (Philipp) 7: 203-216.

33) Usui Y, Nakasa M, Hotta H, Urisu A, Aoki N, et al. (2001) A 33-kDa allergen from rice (Oryza sativa L. japonica) cDNA cloning, expression, and identification as a novel glyoxalase I. J Biol Chem 276: 11376-11381.

34) Becher P (1965) Physical properties of emulsions. In: Emulsions, Theory and Practice 2nd ed., New York: Reinhold Publishing Co. p49-84.

35) Das KP, Kinsella JE (1990) Stability of food emulsions: Physicochemical role of protein and nonprotein emulsifiers. Adv Food Nutr Res 34: 82-146.

36) Kato A, Nakai S (1980) Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochem Biophys Acta 624: 13-20.

37) Nakai S (1983) Structure-function relationship of food proteins with emphasis on the importance of protein hydrophobicity. J Agri Food Chem 31: 676-679.

38) Voutsinas LP, Cheung E, Nakai S (1983) Relationships between hydrophobicity to emulsifying properties of heat denatured proteins. J Food Sci 48: 26-32.

39) Aluko RE, Yada RY (1993) Relationship of hydrophobicity and solubility with some functional properties of cowpea (Vigna unguiculata) protein isolate. J Sci Food Agric 62: 331-335.

40) Philips LG, Whitehead DM, Kinsella J (1994) Modification reactions and protein structure. In: Taylor SL, editor. Structure-function properties of food proteins. New York: Academic Press. p207-55.

41) Petrucceli S, Anon MC (1995) Soy protein isolate components and their interactions. J Agri Food Chem 43: 1762-1767.

42) Hettiarachchy NS, Griffin VK, Gnanasambandam R (1996) Preparation and functional properties of a protein isolate from defatted wheat germ. Cereal Chem 73: 363-367.