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

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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 ischemic 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 (germinate 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 Kjeltec® 2300 Analyzer Unit (Kjel-Foss Tecator, Hoganas, Sweden) after digestion for 1 hour at 420 °C using a Kjeldahl 2006 Digester 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 oxidization 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% bromophenol 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 XI (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 spectrofluorophotometer (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., Gardiner, 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 – 1601, Shimadzu, Kyoto, Japan). Absorbance read at 0 min (T_0) was expressed as EA of protein, while ES was calculated as follows: $ES = T_0 \times \Delta t/\Delta T$, where ΔT is the decrease in turbidity (absorbance) of T_0 in the time interval Δt (10 min), and T_0 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 cm^3/min) was introduced into 5 mL of protein solution (0.2% w/v in 0.05 M phosphate buffer, pH 7.4) 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 FC of the proteins. After 3 min, the volume of foam were determined again and FS was calculated as follows: $FS = V_0 \times \Delta t/\Delta V$, where ΔV is the decrease in volume of foam during the time interval Δt (3 min), and V_0 is the volume of foam at 0 time (soon after introduction stopped).

Statistical analysis

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

main effect was significant) and the differences considered significant at P < 0.05.

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 vari-

um [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 composi-

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.

ance 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 Egg-

tions 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 2: Amino acid composition (mg of amino acid per g rice) of protein isolates from non-germinated/control and germinated long grain rice.

Amino acid	Germination days*								P-value
	0 (control)	1	2	3	4	5	6	7	
Cysteic	1.8 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.5 ± 0.2	1.4 ± 0.0	1.5 ± 0.0	0.1088
ASP	6.4 ± 0.2ab	5.3 ± 0.2d	5.7 ± 0.3cd	5.7 ± 0.2cd	5.7 ± 0.1cd	5.9 ± 0.2c	6.0 ± 0.2bc	6.5 ± 0.1a	0.003
THR	1.9 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.9 ± 0.3	1.7 ± 0.0	1.8 ± 0.1	0.0685
SER	4.3 ± 0.7	3.6 ± 0.2	3.8 ± 0.01	3.6 ± 0.1	3.6 ± 0.4	3.4 ± 0.6	4.1 ± 0.1	4.3 ± 0.01	0.1696
GLU	11.3 ± 0.5a	9.2 ± 0.5b	8.9 ± 0.7b	9.0 ± 0.2b	9.2 ± 0.2b	9.1 ± 0.3b	9.4 ± 0.1b	9.9 ± 0.1b	0.015
PRO	3.4 ± 0.2a	2.7 ± 0.2cd	2.7 ± 0.1cd	2.7 ± 0.1cd	2.8 ± 0.2bcd	3.1 ± 0.2abc	3.2 ± 0.0ab	3.3 ± 0.1a	0.0171
GLY	3.4 ± 0.1a	2.7 ± 0.1e	2.7 ± 0.1e	2.8 ± 0.1de	2.8 ± 0.1de	3.0 ± 0.1cd	3.1 ± 0.0bc	3.3 ± 0.1ab	< 0.0005
ALA	4.0 ± 0.2a	3.2d ± 0.1d	3.3 ± 0.1d	3.3 ± 0.1d	3.4 ± 0.1cd	3.7 ± 0.1bc	3.9 ± 0.0ab	4.1 ± 0.1a	< 0.0003
VAL	3.1 ± 0.2	2.9 ± 0.3	2.8 ± 0.3	3.0 ± 0.5	3.0 ± 0.3	3.0 ± 0.1	2.9 ± 0.0	3.1 ± 0.1	0.9389
ILU	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.3	2.1 ± 0.4	2.1 ± 0.2	2.2 ± 0.2	2.1 ± 0.0	2.2 ± 0.0	0.7982
LEU	5.2 ± 0.2a	4.3 ± 0.2cd	4.3 ± 0.3cd	4.5 ± 0.2cd	4.6 ± 0.3bcd	4.8 ± 0.2abc	5.0 ± 0.0ab	5.0 ± 0.1ab	0.0217
TYR	4.5 ± 0.2a	3.1 ± 0.3c	3.2 ± 0.5c	3.3 ± 0.6c	3.2 ± 0.5c	3.3 ± 0.5bc	4.2 ± 0.1ab	4.2 ± 0.1ab	0.029
PHE	3.2 ± 0.2	2.7 ± 0.2	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	2.9 ± 0.2	3.0 ± 0.0	3.0 ± 0.0	0.1122
MET	1.9 ± 0.5	1.6 ± 0.4	1.9 ± 0.1	1.5 ± 0.3	1.8 ± 0.0	1.7 ± 0.5	2.2 ± 0.0	1.4 ± 0.1	0.3346
HIS	2.8 ± 0.1a	2.5 ± 0.1c	2.6 ± 0.1bc	2.6 ± 0.2bc	2.6 ± 0.0bc	2.7 ± 0.1ab	2.9 ± 0.0a	2.6 ± 0.0bc	0.0119
LYS	7.1 ± 0.1a	6.3 ± 0.0b	6.2 ± 0.3b	6.5 ± 0.4b	6.4 ± 0.2b	7.0 ± 0.2a	7.4 ± 0.0a	7.1 ±0.2a	< 0.0023
ARG	5.5 ± 0.1a	4.2 ± 0.1cd	4.1 ± 0.1d	4.1 ± 0.1d	4.2 ± 0.1d	4.1 ± 0.1d	4.5 ± 0.0b	4.4 ± 0.0bc	< 0.0001

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

group. Glutamic acid (P = 0.0150) content was the highest in control 11.3 mg/g rice and the lowest was at 2 day of germination. Glutamic acid is a key molecule in cellular metabolism and plays an important role in the body's disposal of excess or waste nitrogen. The main process in amino acid degradation is transamination. Transamination effectively allows nitrogen from the amine groups of amino acids to be removed, via glutamate as an intermediate, and finally excreted from the body in the form of urea. In food products glutamic acid is used as food additive and flavor enhancer.

Proline had similar pattern as aspartic acid that decreased at 1 day of germination and increased at 7 day of germination making it significantly different from the control (P = 0.0171). Proline is considered to be unique because the α-amino group is secondary. Glycine content (P < 0.0005) of the control was 3.4 mg/g rice and decreased at 1 day of germination to 2.7 mg/g rice. In food products, glycine is used as sweetener or taste enhancer.

Alanine content (P < 0.0003) of the control (4.0 mg/g rice) showed slight difference in comparison with germinated rice at 7 days (4.1 mg/g rice). Lasztity and Hidvegi reported alanine content in brown rice was 5.6 mg/g rice [31]. This slight difference in values might be due to variations in germination procedure or the type of rice used for the analysis. Alanine takes part in glucose-alanine cycle between tissues and liver. In this study valine contents of the control and germinated rice at 7 days are the same (3.1 mg/g rice) but were not significantly different with the other germinated rice samples (P = 0.9389). Similarly, the isoleucine content of the control (2.0 mg/g 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 = 0.7982). Along with valine, leucine and isoleucine are branched-chain amino acids. Juliano [26], Eggum et al. [27], and Pedersen and Eggum [28] reported valine, leucine and isoleucine contents in rough rice ranging from 4.6 - 7.0, 6.9 - 8.8, and 3.0 – 4.8 mg/g rice, respectively.

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 food and drink products and used as nutritional supplements 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 to 1.4 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 d 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.

lergenic [33]. In this study, the electrophoretogram indicated disappearance of allergenic proteins with molecular masses of 33 and 56 kDa at 6 and 7 days and 26 kDa at 5 to 7 days. The decrease in high molecular weight proteins and an increase in low molecular weight proteins were observed as germination progressed which can be explained on the basis of specific proteases hydrolyzing high molecular size proteins to low molecular size proteins (peptides).

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

Figure 1: Electrophoretogram of rice protein isolates from non-germinated/control and germinated long grain rice
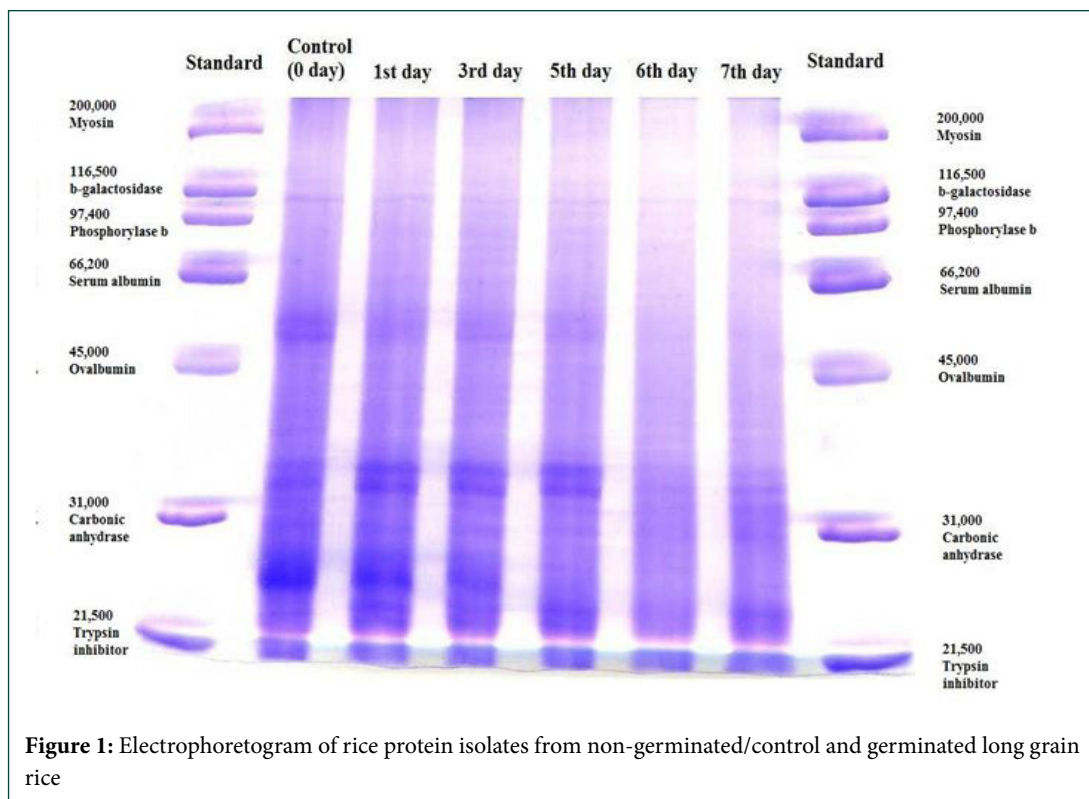
Cereal proteins are known to cause allergic disorders such as asthma, eczema and dermatitis, and gluten-sensitive enteropathy. In rice, proteins with molecular masses of 13-16, 26, 33, and 56 kDa have been reported to be potentially al-

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 from1 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.

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

Table 3: Surface hydrophobicity, emulsifying and foaming properties of protein isolates from non-germinated/control and germinated long grain rice.

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

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 cohesiveness, 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

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